The effect of foliar applied fertilisers on leaf diseases of cereals

Thesis

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THE EFFECT OF FOLIAR APPLIED FERTILISERS ON LEAF DISEASES OF CEREALS.

JOHN WILLIAM COOK

B.A. (Hons.) University of Oxford

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Crop and Environment Research Centre

Harper Adams Agricultural College

Newport

Shropshire

TF10 8NB

UK

THE EFFECT OF FOLIAR FERTILISERS ON LEAF DISEASES OF CEREALS.

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Abstract

The effects of foliar applied urea and potassium chloride on the severity of leaf diseases of cereals were investigated in the laboratory, glasshouse and field between 1992 and 1995. Field studies with urea gave inconsistent results with respect to severity of *Erysiphe graminis* and consistently increased the leaf area affected by *Septoria tritici*. However, potassium chloride applied as a foliar spray consistently decreased the leaf area of wheat affected by *E. graminis* and *S.tritici* compared with equivalent applications of soil applied fertiliser. Disease control was achieved at early stem extension and after flag leaf emergence but yield responses were not detected. Laboratory investigations were undertaken to determine the mechanism by which foliar applied potassium chloride reduced the leaf area affected by *E. graminis*. The timing of application, within seven days pre or post inoculation, had no consistent effect on the efficacy of the fertiliser. Investigations using polyethylene glycol as a control showed that the percentage leaf area affected declined linearly as the osmotic potential of the solutions were increased. Light microscopy revealed that the germination of spores in solution and on treated leaves was reduced as the osmotic potential of the solutions were increased. Spores which did germinate developed normally but those on leaves treated with solutions of high osmotic potential rarely formed haustoria. This suggested a second mechanism acting inside the leaf. Multiple regression analysis of experimental data indicated that the inhibition of spore germination was the major response reducing the area of the leaf affected. Although the data were not conclusive it appeared that the increase in leaf water potential, following the foliar application of potassium chloride, was involved in the control of *E. graminis*.

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Chapter 1 - Introduction

1.0 Rationale

The application of fertiliser solutions, as foliar sprays, to the leaves of cereal plants has been shown to reduce the leaf area of cereal plants affected by foliar disease in the field (Gooding, M.J., Kettlewell, P.S. and Davies W.P., 1989; Kettlewell, P.S., Bayley, G.L., and Domleo, R., 1990). There have been few reports of laboratory experimentation regarding this phenomenon and information relating to the mechanism by which foliar fertilisers reduce disease severity are very limited.

The experiments reported in the literature had no control treatments where fertiliser was applied to the soil simultaneously with the application of foliar treatments. Therefore it was not known whether the disease control was attributable to a nutritional effect, direct toxicity, or some physiological changes in the leaf unrelated to nutrition.

The application of fertilisers to the leaf surface was thought likely to cause significant alterations in the environment encountered by pathogenic, saprophytic and commensal phylloplane micro-organisms. It was suspected that the application of a nutrient solution would cause a change in the osmotic environment and the balance of available nutrients on the leaf surface favouring some of the microbes and inhibiting others. Direct toxicity of some fertiliser compounds to certain species was also considered possible. These factors, coupled with major changes in leaf physiology as a result of fertiliser application, might possibly favour some species of microbe and inhibit others resulting in population changes in the microbial population of a leaf. It was considered that the application of foliar fertilisers could be used to deliberately manipulate the leaf environment and render it unfavourable to the pathogens of cereal plants.

There would be immense benefits from such an approach to crop protection. If disease was reduced below the threshold for fungicide application there would be environmental and commercial benefits. Environmental benefits would result from less energy being used in crop production and reduced release of highly toxic compounds into the environment. Commercial benefits would result from reduced expenditure on fungicides and possible price premiums for cereals produced without the use of conventional fungicide.

1.1 Aims

The project had three aims. Firstly, to identify a foliar fertiliser which reduced the severity of a foliar cereal disease. The effectiveness of this fertiliser would then be tested in the field and under controlled conditions in the glass house and laboratory. The final objective was to elucidate the mechanism by which the fertiliser reduces disease severity. It was decided at the beginning of the project that any compound used would have to prove effective in spray volumes in the region of 200 l/ha as the cost of applying greater volumes would prove more expensive than current fungicide application costs.

The initial approach to the problem was a thorough review of the literature on foliar fertilisers and cereal disease followed by a more specific review of crop nutrition and disease. The latter review concerned only elements contained in foliar fertilisers giving disease control.

Promising fertilisers identified by literature review would then be applied at intervals throughout the growing season to challenge naturally occurring disease epidemics in field experiments. The most effective of these fertilisers would then be evaluated in the glasshouse against one of the diseases which it controlled in the field. The final stage would be the investigation of the mechanisms by which the chosen fertiliser acted against a specific disease.

1.1.1 Cereal Disease in Relation to Foliar Applications of Fertiliser

A thorough literature review of crop nutrition and disease revealed a limited number of reports on the control of fungal diseases on cereals by foliar applied fertiliser solutions. Most of the information related to soil applied fertilisers and soil borne diseases. Much of the information had little relevance to the rationale of the project and was therefore not included in this review.

Potassium chloride has been shown to reduce the symptoms of infection of wheat by *Septoria nodorum* Murrat (Kettlewell *et al.*, 1990) and of barley by brown rust *Puccinia hordei* Rob and Desm (Kettlewell, Blouin and Boulby, 1992). The latter observation contrasts with that of Hashim and Russell (1982) who concluded that only soil applied chlorides affected levels of brown rust infection on cereals. Russell (1978) also reported that soil, but not foliar applied, sodium chloride reduced the severity of yellow rust, *Puccinia striiformis* West., on wheat. Hashim and Russell (1982) and Russell (1978) used only one concentration of sodium chloride in their experiments. By using only one concentration of this salt solution it would seem that they did not fully evaluate the potential of foliar applied chloride salts. They may have missed a critical concentration of sodium chloride capable of controlling *Puccinia* spp. Equally they neglected to investigate the possibility that the control of *Puccinia* species by foliar applied chloride salts may also be dependent upon the cation in the solution.

It has been shown that applications of foliar nitrogen to the flag leaf as a urea solution can reduce the levels of powdery mildew (*Erysiphe graminis)*, *Septoria nodorum* and *Septoria tritici* Rob and Desm in the field (Gooding *et al.*, 1989). Other workers reported inconsistent reductions of mildew and *Septoria* in the field (Penny, Widdowson and Jenkyn, 1978). The control of *S. nodorum* with foliar urea

in the field and in glasshouse studies has also been reported (Peltonen, Kittila Peltonen-Saino and Karjalainen, 1991). These workers found no reduction in leaf area affected by *S. nodorum* when the urea was applied after inoculation and suggested that urea reduced disease severity by inhibiting infection when applied before inoculation. It was indicated that concentrations of five percent or more would prove toxic to *S. nodorum* grown on agar media.

It was not clear whether these reductions in apparent disease were due to direct toxicity to the pathogen, as indicated by Peltonen *et al.* (1991) with respect to *S. nodorum* or due, at least in part, to complex nutritional effects on host plant resistance. This work was done with plants which had surplus tillers removed and it was possible that this may have modified the response of the host to fertiliser applications. Using electron microscopy Peltonen *et al.* (1991) found that urea reduced the severity of *S. nodorum* by inhibiting pathogen development before leaf penetration occurred. With respect to mildew it has been suggested that the effect of urea was due to sub-clinical levels of leaf scorch affecting the host plant cell membranes and therefore affecting the host-pathogen interaction or recognition (Penny *et al.*, 1978).

On the basis of the limited evidence provided by Kettlewell *et al.*, (1990) Gooding *et al.* (1989) Penny *et al.* (1978) and Peltonen *et al.* (1991) it was decided that the best lines of investigation were the interactions of the diseases powdery mildew, *Septoria tritici* and *Septoria nodorum* with urea and potassium chloride fertilisers.

It was decided to review the general relationship between crop nutrition and disease followed by specific reviews of nitrogen, potassium and chloride.

1.1.2 The Influence of Nitrogen, Potassium and Chloride Nutrition on Foliar Diseases of Wheat and Barley.

The nutrition of cereal crops has a very significant influence on the morphology and physiology of their constituent plants. Manipulation of the available nutrient supply affects both crop yield and quality. By altering the physiology and morphology of the cereal plant, nutrition can play a significant role with respect to its resistance or susceptibility to disease. Physiological changes affect pathogenesis by affecting the susceptibility of the host to infection and the rate of development of the pathogen in plant tissues. Morphological changes alter the micro-climate of the crop as experienced by the pathogen and may therefore facilitate or impede germination and dispersal of propagules into the environment. A deficiency of nutrient elements may be due to an absolute shortage in the environment, environmental conditions inhibiting uptake or pathogenic infection causing redistribution in the plant tissues. Any of these conditions can influence disease development. The availability of certain elements in the crop environment or in the plant can sometimes facilitate the development of diseases. Elements are directly involved in both passive and active mechanisms of the defensive systems of plants, as structural cell components, enzymes and electron carriers, or as activators, elicitors, inhibitors and regulators of metabolism.

Elements and ions in the soil or plant tissues are part of the environment encountered by microbial pathogens and soil micro-flora. In consequence the response of pathogens and competitive non-pathogenic organisms to nutritionally induced changes in the rhizosphere and phylloplane may also influence the occurrence and severity of disease. The complexity of the interaction between diseases and crop nutrition is compounded by the frequent observation that the effect of a particular element on cereal diseases was often dependent upon the anion or cation with which it was applied (Huber, 1980; Powelson, Jackson and Christensen, 1984). This

indicates the need to consider the role of each element individually so that the effects of the fertilisers on crop disease may be more fully understood.

1.1.3 Nitrogen

Nitrogen is considered one of the most important elements contained in fertilisers. It is commonly deficient in most agricultural soils and frequently applied to increase yield and protein content of cereal crops. Nitrogen is an essential element for the production of amino acids, proteins, growth hormones, new protoplasm, phytoalexins and phenols. The application of nitrogen to deficient crops has been observed to promote vigorous growth, delayed maturity, increased cell size and decreased cell wall thickness. All of these factors influence a plant's susceptibility to pathogenic attack. Consequently the study of nitrogen in relation to foliar disease of plants has been and still is relatively intensive.

The interaction of nitrogen nutrition and the pathogen *Erysiphe graminis* DC. has been the subject of much study. *E. graminis* causes powdery mildew disease of cereals. The powdery mildews are considered to be some of the most economically important pathogens in the world and they have a particular importance in the grain growing regions of Western Europe. Although the high yield of commercial cereal crops depends to a large extent on the application of nitrogenous fertiliser, nitrogen tends to increase the severity of mildew infection (Jenkyn, 1977; Jenkyn, Finney and Dyke, 1983; Last, 1953; 1954; 1962a; Daamen, Winjnands and van der Vliet, 1989; Darwinkel, 1975). However, this is not always the case and the application of nitrogen containing fertiliser has been shown to decrease mildew on at least one occasion at Rothamsted Experimental Station, Harpenden, UK (Jenkyn and Moffatt, 1975). The relationship between nitrogen fertiliser rate and the incidence of powdery mildew is very complex. Most workers report increases in the incidence of powdery mildew with increasing application rates for nitrogen. This effect may be

due in part to changes in crop density and hence micro-climate. It is generally believed that high applications of nitrogen result in a lush dense canopy leading to increased humidity facilitating higher spore germination. It has also been shown that increasing nitrogen nutrition of the host increased the growth and sporulation of the fungus excluding canopy effects (Bainbridge, 1974). Therefore applying nitrogen fertiliser to cereal crops results in increased host susceptibility, a more favourable environment for spore germination and increased inoculum production.

Jenkyn *et al.* (1983) indicated that nitrogen applied at rates up to 90 kg/ha increased mildew in 1975 and 1976 but decreased it above these rates. They postulated that under the very dry prevailing weather conditions large quantities of nitrogen may act to inhibit mildew (Jenkyn and Finney, 1981). There was no indication of the reason why this occurred but the application of large quantities of nitrogenous fertiliser may have resulted in a lush canopy with a high transpirational capacity in which individual plants were subjected to high water stress. This may have resulted in physiological changes in the plants, particularly an increase in the osmotic potential of the cytoplasmic solution, which rendered them less susceptible to infection by *E. graminis*.

The interaction between nitrogen and *E. graminis* is particularly interesting because of the effect which *E. graminis* exerts upon the metabolism of nitrogen in the plant.

Barley leaves infected with *E. graminis* f.sp. *hordei* have been shown to accumulate ammonium ions, probably due to infection reducing the ability of the leaf to incorporate amino acids into protein by limiting the supply of 2-oxyglutarate (Sadler and Scott, 1974). Infected plants also liberated appreciable amounts of ammonia gas which could have accelerated the metabolic disruption which occurred in the affected leaves. These workers found no consistent changes in the nitrite or nitrate content of the barley tissues four days after infection. It was reported that

seven days after infection all tissues of diseased plants exhibited a lower nitrate content than the comparable tissues of uninfected plants. The tissues, with the exception of the youngest unfolded leaf, also showed increased concentration of ammonium ions while the nitrate content of both the roots and leaves was lowered. This was ascribed to reduced uptake of nitrate by the roots which was later confirmed by Jenkyn (1977) who showed that infection of cereals by powdery mildew reduced the nitrate content of bleeding sap collected from the roots. This could in turn explain why the leaf nitrate concentration of the infected plants was lowered since nitrate is one of the major nitrogenous materials translocated to the shoot (Pate, 1973).

Nitrate uptake may be lowered by the accumulation of ammonium ions in the root (Hewitt, 1975) or, as is more likely, because the root was starved of photosynthetic products (Walters and Ayres, 1983), since mildew is known to reduce the translocation of metabolites to the roots (Lupton and Sutherland, 1973). These carbohydrates are essential for the uptake of nitrate by the root. The implications of this with regard to the optimal nutrition of infected crops are unclear since the plants are overloaded with ammonium in the tissues despite overall nitrogen uptake being reduced. This was further complicated because in some cases disease has been shown to affect photosynthesis more than nitrogen uptake, especially in stem tissue, resulting in the accumulation of nitrate as a result of mildew infection. This was demonstrated in pot experiments (Jenkyn, 1977) but this was a very artificial situation and root development, which was dependent upon translocated carbohydrate, was probably of less importance than in the field.

Nitrogen fertiliser can influence the adult plant resistance of cereals to foliar disease. Last (1954) showed that adding nitrogen to deficient wheat plants caused already mature leaves which had resisted mildew infection to become susceptible. This nitrogen-induced increase in susceptibility to powdery mildew infection was

temporary in the older leaves. The percentage conidial germination and appressorium formation on resistant and susceptible leaves did not differ, suggesting that the nitrogen induced physiological rather than leaf surface changes. Last also found that late applications of nitrogen fertiliser increased the number of pustules and cleistothecia formed thus increasing the likelihood of carry over of the disease. On spring barley it was found that nitrogen applied late increased disease severity compared to nitrogen applied earlier (Jenkyn *et al.*, 1983). This contrasted with the findings of other workers. Nitrogen top dressings applied in March to winter barley in the United Kingdom resulted in more foliar diseases including mildew than later applications (Jenkyn, 1977) indicating that the timing of fertiliser applications can influence the severity of disease symptoms.

Diseased crops produce less dry matter than healthy ones and so might be expected to have a smaller total nitrogen demand but it does not follow that less nitrogen need be applied (Jenkyn and Finney, 1981). These workers suggested that the root systems of plants affected by powdery mildew are smaller than those of healthy plants (Last, 1962b; Paulech, 1969) and are therefore less efficient at capturing nitrogen leached down the soil profile.

The effect of nitrogen fertiliser practice on the severity of rust (*Puccinia* spp.) diseases is unclear. Some workers claim that nitrogen increases the severity of rust diseases (Huber and Watson, 1974) while others have reported little effect (Das and Sen, 1975). Daly (1949) and Huber and Watson (1974) agreed that black rust (*P. graminis*) of wheat was increased by the application of nitrogen but that the response was dependent upon the form of nitrogen used, with nitrate being more favourable. It was generally accepted that high levels of nitrogenous fertiliser produced very lush canopies and this resulted in the creation of a micro-climate more favourable to the leaf infecting rusts. Increasing the nitrogen concentration in the soil by applying nitrogen fertiliser decreased the number of lesions on pot grown barley plants, caused

by leaf blotch (*Rynchosporium secalis*), suggesting a physiological effect which rendered the host tissue more resistant to invasion (Jenkyn and Griffiths, 1978). This contrasts with the increased leaf area affected by *R. secalis* where nitrogen fertiliser was applied in the field (Jenkyn, 1969 from Jenkyn, 1977). This divergence in findings suggests that the increases in leaf blotch of barley commonly associated with high rates of nitrogen fertilisation are due to changes in the micro-climate of the crop. It appears likely that the dense canopy produced by nitrogen fertilisation provides a more humid environment and consequently higher spore germination than the canopy of unfertilised crops. The divergence of the findings indicated the need to investigate plant nutrition interaction with disease in both the laboratory and field situation.

Singh (1961) studied the effect of mineral nutrition deficiencies and the effect of different levels of mineral nutrients on the net blotch pathogen of barley (*Pyrenophera teres* Drechsler). Nitrogen concentrations below that of the control solution in hydroponic culture were found to decrease the severity of disease. In an experiment relating the concentration of nutrient to the severity of disease, increasing the element in the solution resulted in increased disease severity. In this case it appeared that tissues with an increased nitrogen content provided a superior substrate for the pathogen. The differential responses of *P. teres* and *R. secalis* indicates how the interaction of nitrogen nutrition and disease differs between pathogens.

In the case of soil-borne root and stem base infecting fungi it generally appears that the quantity and type of nitrogen fertiliser applied has a great effect upon the severity of disease symptoms (Huber and Watson, 1974). This is not the general case with foliar cereal diseases. In general foliar disease interactions with host nutrition are largely related to the quantity of nitrogen available. However this may have been because quantity rather than form of fertiliser was the major variable in most of the experiments examined. The interaction of foliar diseases with type of

nitrogen fertiliser applied is potentially a very important area of research. However certain generalisations can be made. With respect to necrotrophic pathogens it appears that field and laboratory experiments frequently yield conflicting results. In the case of both *S. tritici* and *R. secalis* increased application of nitrogen appeared to reduce the leaf area affected where plants were grown singly. However this situation tended to be reversed in the field where nitrogen increased the volume of above ground biomass leading to greater canopy density and altered the microclimate. This resulted in conditions which were more suitable for infection and produced results which were the reverse of those obtained in the laboratory investigations. It is possible that high applications of nitrogenous fertiliser produced plants which individually have greater resistance to necrotrophic pathogens, possibly through enhanced production of defensive chemicals such as phenols, but that this is negated by the superior environment for infection at the community level created by larger, more dense canopies. It appears that the interactions of nitrogen nutrition and cereal disease are very complex and any one strategy will have both positive and negative results. It would seem that nitrogen fertiliser policy could be used to manipulate certain diseases as part of an integrated crop management policy. However, in view of the high yield responses to nitrogen fertiliser by small grain cereals, such a move would have to be very carefully evaluated. It would appear that changing the form of the fertiliser rather than the total quantity of nitrogen may be able to decrease disease severity without reducing yield.

1.1.4 Potassium

Disease interactions with potassium are particularly noted with respect to the biotrophic pathogens such as cereal rusts and powdery mildew. Glynne (1958) reported decreases in the incidence and severity of powdery mildew in response to potash applications especially when nitrogen and phosphate were supplemented at the

same time. Powdery mildew was also reported to be reduced by potassium chloride applications but not by calcium chloride, indicating that these reductions were due to potassium and not the associated chloride anion (Grybauskus and Sammons, 1988). However, Last (1962a) reported no reduction in response to applications of potassium. It appeared that the response to potassium fertilisation was dependent upon the relative supply of other nutrients to the plant.

Potassium and brown rust interactions are widely studied. It is reported that the infection of barley with brown rust resulted in increased uptake of potassium sixteen days after inoculation (Ahmad, Owera, Farrar and Whitbread, 1982) which resulted in major alterations of the nutrient balance in the leaf. This illustrates the influence which pathogens can have on host-plant nutrition.

The effect of chloride on brown rust (*P. recondita*) infection is reported elsewhere but it has been noted that the application of potassium chloride to the soil suppresses brown rust more effectively than other chlorides. When applied to the foliage, Hashim and Russell (1982) reported that sodium chloride had no effect upon the severity of brown rust. This contrasts with the reductions in severity obtained with the foliar application of potassium chloride by Kettlewell *et al.* (1992). The reduction in the incidence of rust by potassium is not limited to *Puccinia recondita* and application of potassium fertilisers has been widely quoted as a cultural control method to reduce the severity of *Puccinia* spp. and may partially offset the rust-enhancing application of nitrogen fertiliser (Butler and Jones, 1949).

Temiz (1977) reported that high levels of potassium applied in sulphate form resulted in the formation of larger pycnidia of *S. tritici* but that total number of pycnidia and the size of the lesions on the leaves was reduced. It was suggested that potassium sulphate reduced the overall severity of the disease by interfering with the infection process. However some doubt remained as to which ion, potassium or sulphate, was responsible for the control. Kettlewell *et al.* (1990) indicated that

foliar-applied potassium chloride reduced the severity of *S. nodorum* on wheat when applied late in the season. *S. tritici* was apparently unaffected. The severity of the net-blotch disease of barley (*Pyrenophera teres)* was increased by the application of potassium fertiliser in proportion to the quantity applied (Singh, 1961).

Potassium is considered a mobile regulator of cellular activity and a key component of many plant enzyme systems (Liebhardt, 1968). The concentration of potassium in a plant depends upon the availability of calcium and magnesium in the environment which is in turn dependent upon the pH. Potassium availability was enhanced by the presence of calcium in neutral soils but not in acidic soils. A deficiency of potassium impairs the utilisation of phosphorus and nitrogen in a manner similar to a true deficiency (Huber and Arny, 1985; Huber, 1978). The mechanism by which potassium exerted its influence was unclear. It was suggested that the key role which potassium played in nitrogen metabolism may result in the accumulation of organic nitrogen in the plant sap (Liebhardt, 1968). This organic nitrogen was possibly less available to the fungi than the synthates otherwise available under potassium deficient conditions and may have consequently reduced disease severity. As indicated with respect to *Septoria*, the effects of potassium are not always beneficial and a proportion of its reputation as "the plant health element" may be due to the unrecognised effects of the associated anion, particularly chloride. The benefits of potassium fertilisation with regard to plant health appear to occur when it was applied to correct a deficiency. Frequently these deficiencies are induced by the application of disproportionate amounts of nitrogen or phosphorus fertiliser.

1.1.5 Chloride

Chloride is implicated in the suppression of cereal diseases on several continents and is especially well researched in the Pacific North West of the U.S.A. This element has been implicated in the reduction of disease severity of brown rust

(*Puccinia recondita*), powdery mildew (*Erysiphe graminis*) and yellow rust (*Puccinia striiformis*).

The effect of chloride on the rust genus *Puccinia* has been noted but there is considerable disagreement in the literature. Christensen *et al.* (1982) reported that yellow rust was reduced on 381 cultivars of wheat by the application of ammonium chloride. This evidence was supported by laboratory studies by Russell (1978) who observed reductions in yellow rust severity in response to applications of sodium and potassium chloride in pot grown plants and field plots of wheat. Powelson *et al.* (1984) also reported yellow rust reductions in response to chloride application.

Chloride was also reported to reduce the severity of brown rust when applied to the soil (Hashim and Russell, 1982). However there was an interaction between forms of chloride with potassium chloride giving greater reductions than lithium or sodium chloride. Russell (1978) attributed the control of rust with chloride fertiliser to the modification of the rhizosphere environment and changes in the microbial population leading to reduced nitrification and reduced nitrate uptake. Modification of the nitrate/ammonium uptake ratio can have far-reaching consequences on the host physiology and pathogen response as described above. There is some debate about the effect of foliar-applied chloride on rust. Russell (1978) and Hashim and Russell (1982) maintained that both yellow and brown rusts were not controlled by foliar applications of chloride. However these workers used only sodium chloride as a foliar spray. Kettlewell *et al.* (1992) contested this observation and reported the control of barley brown rust (*P. recondita* fsp. *hordei*). It seems probable that foliar applied chloride fertilisers have different physiological effects upon the host than soil applications which may explain the difference in the findings of different workers. Alternatively the differences in the findings may be due to the potassium ion being the essential element in this effect.

Applications of chloride fertiliser were implicated in the control of *Septoria* spp. by Powelson *et al.* (1984). Kettlewell *et al.* (1990) showed that *S. nodorum* but not *S. tritici* was controlled by the application of foliar potassium chloride in the field.

Powdery mildew control by the application of chloride salts has been reported by several workers. Grybauskus and Sammons (1988) reported that the application of chloride fertilisers to the soil reduced the area under the powdery mildew disease progress curve but only when applied as potassium chloride and not when applied as calcium chloride. This suggested that the cation was more influential than the chloride ion in this case. However Kettlewell *et al.* (1992) found that foliar applications of potassium chloride reduced powdery mildew on barley, albeit at very low levels, but not on wheat (Kettlewell *et al.*, 1990).

Various mechanisms were proposed for the activity of chloride which results in the reduction of plant disease. Two of these mechanisms relate to the effect of chloride on nitrogen nutrition and metabolism. The application of chloride salts increases proline and betaine synthesis at the expense of other nitrogen compounds which may be more available to the pathogen (Murray and Ayres, 1986). These workers thought that this was the mechanism by which chloride salts reduced infection by *E. graminis* and it appeared logical that such an effect may also have been involved in the reduction of other pathogens especially biotrophs such as the rusts.

Chloride is also believed to affect the soil microflora and to inhibit nitrification resulting in modification of the ammonium to nitrate ratio with the associated effects on disease incidence reported above. Chloride may affect the establishment of pathogens inducing changes in the osmotic potential of the cells and in turn affect the suitability of the plant as a substrate for fungal growth. On the whole it would appear that the application of chloride-containing fertilisers is

responsible for many changes in both the soil microbial population and the physiology of crop plants.

1.1.6 Discussion

Nutrition appears to have a very significant influence on cereal disease. It is apparent that the responses of individual pathogens to changes in host nutrition are very different. It is difficult to draw general conclusions about the response of cereal disease to changes in nutrition or identify fertiliser strategies which will reduce disease of cereals overall.

In many cases the effect of fertiliser applications are due as much to induced changes in the environment as strictly nutritional properties. An example of this is the application of lime which provides calcium to the plant and also reduces soil acidity. Reducing soil acidity favouring nitrification alters the ammonium : nitrate ratio of the soil with important consequences for disease severity.

The only general trend apparent in the literature is the importance of balanced nutrition. Many of the deleterious effects occurred when one element was increased in isolation. Much of the information relating to take-all and powdery mildew followed this pattern which was reversed when all nutrients were increased simultaneously. It appears that well fertilised plants were generally more vigorous and healthy than nutritionally deficient plants. When considering the balance of nutrients available to the plant it is clear that in many cases the effect of one nutrient is dependent upon the availability of another. This is particularly evident with chloride which appears to exert many of its effects by altering the form of nitrogen available to the plant and its subsequent metabolism.

Overall it appears that the subject of crop nutrition and cereal disease has been inadequately researched. Much of the available literature related only to disease

responses where one element is varied at a time. There is a scarcity of literature relating to the mechanisms by which nutritional changes alter disease severity and an absence of information regarding the ideal ratio of available nutrients for the health of cereal plants. However it was clearly evident that manipulation of cereal diseases by nutritional means could become a significant tool in the integrated management of crop production. If this could be achieved without major changes in crop yield and quality such a move might have significant commercial and environmental benefits.

1.2 Foliar Fertilisers

For the purposes of this project, foliar fertilisers were defined as nutrient solutions applied as a droplet spray directly to the leaves of crops rather than to the roots via the soil. A proportion of the foliar fertilisers applied to crops have been observed to be intercepted by the leaves and absorbed directly. Fertilisers applied by this method could therefore be taken up when the soil is too dry for conventional fertilisers to be dissolved and move down the soil profile to the roots. This means that foliar fertilisation can be carried out throughout the growing season. It was suggested that by applying fertilisers at critical times for the crop without regard for soil moisture status the yield and quality of crops could be improved (Eibner, 1985). Furthermore by applying fertilisers when the crop needs them rather than when the soil was moist in the spring, the potential for nutrient leaching is reduced. This is considered to have environmental benefits by reducing pollution of ground water and potential commercial benefits since it would permit a reduction in the quantity of fertiliser applied with conventional strategies.

1.2.1 Urea

Urea or carbamide is manufactured by the reaction of anhydrous ammonia and carbon dioxide under conditions of high temperature (170 to 230 $^{\circ}$C) and

pressure (120 to 280 bar). The resulting product is further processed to produce a white highly soluble powder containing 46% nitrogen (Quarmby, 1991). It is frequently used for making fluid fertiliser either as the sole nitrogen source or with other fertilisers. As a foliar spray urea is most widely used as a late season spray to improve the protein content of wheat for bread making although application pre anthesis can increase yield but not protein content (Gooding, *pers com*).

Experience revealed urea to dissolve with a strong endothermic reaction and the process was heat input limited. Indeed it is not uncommon for suspensions to freeze during mixing. If urea is added to water quickly the solution will freeze. In one experiment it was found that visible phytotoxicity manifested as scorch was slight from solutions more dilute than ten percent (Kettlewell and Juggins, 1992).

1.2.2 Potassium Chloride

Potassium chloride (KCl), a naturally occurring fertiliser sold as muriate of potash, is commercially extracted from both brine and deposits of the mineral sylvanite (Kapusta, 1968). Most samples contain sixty percent dipotassium oxide (K_2O) equivalent. Potassium chloride is extracted from sylvanite by both flotation benefication and solution re-crystallisation. The latter process is used for exploiting naturally occurring subterranean brine lakes. The resulting salt is then broken to a white powder and dissolved to make a fluid fertiliser or granulated for easy handling as a solid. Potassium chloride solutions are not usually applied as a foliar spray to cereals but yield increases have been achieved by an application around flag leaf emergence on wheat (Kettlewell *et al.*, 1990).

1.3 Pathogens

1.3.1. *Erysiphe graminis*

E. graminis DC., causal agent of the cereal powdery mildew disease, is a biotrophic ascomycete fungus which produces white superficial hyphae on the living aerial parts of the host plant, with large one-celled conidia produced terminally on isolated unbranched conidiophores. Lobed haustoria are produced in the epidermal cells of the host plant. Observation of older colonies has revealed colourless one celled ascospores borne in asci enclosed in black non-ostiolate perithecia. Conidia have been observed to be passively dispersed by wind (Jenkyn and Bainbridge, 1978).

Physiologic specialisation is evident with formae speciales classified on grounds of host specificity. *E. graminis* f.sp. *tritici,* which attacks only wheat, produces smaller more discrete pustules than the other form species.

Infection by conidia or ascospores is by direct penetration of the cuticle by an infection peg. Powdery mildews can tolerate very dry conditions although spore germination increases up to one hundred percent relative humidity (Manners and Housain, 1963; Zaracovitis, 1966) but free water is detrimental. Germination is best at around 10-15°C but Manners and Housain (1963) reported germination at temperatures in excess of 30°C. However at high temperatures germination was much reduced. During germination two germ tubes are produced. At the end of one of these an appressorium forms from which an infection peg grows and penetrates the host cuticle and epidermal cell before producing a lobed elliptical haustorium.

The haustorium, separated from the hyphae by a septum (Bracker, 1968), projects into the cell wall of the host cell causing an invagination of the host plasmalemma but is separated from direct contact, except possibly at the tips of the haustorial lobes, by a matrix of pectic and hemicellulosic substances called the haustorial complex (Gil and Gay, 1977). The extra haustorial membrane is isolated from the rest of the plasmallema by the secretion of a collar of material resembling a

casparian strip (Bracker, 1968). It is proposed that the extra haustorial membrane is semi-permeable but possesses no enzymes for active transport (Spencer-Phillips and Gay, 1981). These workers suggested that a high ATP-ase activity on the haustorial membrane indicates enzyme mediated active transport of substrate from the extra haustorial matrix creating a diffusion gradient which would be replenished by influx from the host cytoplasm. An alternative suggestion by Manners (1979) that the translocation is by active transport was also noted.

Conidia are produced from the mycelial mat. These conidia are known to be capable of causing new infections. The ascospores produced by old colonies are released after the cleistothecium or perithecium imbibes water. In humid climates the ascospores released in the autumn infect volunteer cereals. In colder or drier climates the ascospores over-winter protected by the cleistothecium.

1.3.2 *Septoria nodorum*

Septoria nodorum Berk., the causal agent of glume blotch of wheat and barley, was found to be the imperfect stage of the ascomycete fungal pathogen *Leptosphaeria nodorum* Muller. It is observed on leaves causing lesions up to 1 cm long. These lesions are yellow and elliptical in shape at first, later turning brown and becoming irregular in shape. The lesions often develop purple margins and coalesce. Pinky brown pycnidia then appear on the dead tissue. The pycnidia sporulate and extrude spores in a gelatinous cirrus under conditions of high humidity. The spores are reported to be prevented from germinating under dry conditions by the proteins and glucides which constitute the cirri (Fournet, 1969). The dispersal of spores is by rain splash. The minimum amount of rain needed to disperse the pathogen was reported to be 5mm at a temperature of 10°C (Jordan and Tarr, 1977). The conidia germinate in free water at temperatures between 5 and 37°C. (optimum approx 20-25°C) in the presence of free water (Fournet, 1969).

The germ tubes penetrate the leaf and the mycelium grows, progressively destroying the leaf tissue and reducing the photosynthetic area and hence yield. The pathogen also attacks the ear causing massive yield losses due to shrivelling of the grain (Jenkins and Morgan, 1969).

1.3.3 *Septoria tritici*

Septoria tritici Rob and Desm. is the imperfect stage of *Mycosphaerella graminicola* (Fuckel). The imperfect stage, the causal agent of the leaf blotch disease of wheat, is reported capable of significantly reducing yield (Williams and Jones, 1972). The epidemiology of the disease is similar to that of *S. nodorum.* The conidia are spread by rain splash (Jordan and Tarr, 1977) and germinate in free water to produce irregular yellow spots which enlarge and frequently coalesce. The dead necrotic leaf tissue in the centre of the lesion becomes greenish brown and covered by numerous black pycnidia which extrude white cirri. The disease appears to have cardinal temperatures similar to those for *S. nodorum* for both conidial germination and mycelial growth (Gheorgies, 1974).

Chapter 2 - General Materials and Methods

2.0 Plant Materials and Cultivars

2.0.1 Wheat

For the purposes of this research three cultivars were used. Two cultivars were soft endosperm texture, red-grained winter feed wheats which differed in origin and disease susceptibility.

Cv. Apollo is a German bred variety (Breun, Germany; U.K. distributor New Farm Crops, Lincolnshire) which is very susceptible to powdery mildew disease caused by *Erysiphe graminis* and moderately susceptible to glume and leaf blotches caused by *Septoria nodorum* and *Septoria tritici* with N.I.A.B. disease resistance ratings for powdery mildew 3; *Septoria tritici 7*; *Septoria nodorum* 5 (Anon, 1994b). N.I.A.B. disease resistance ratings are on a relative scale with 1 being extremely susceptible and 9 being resistant. Cv. Riband is an English bred variety (Plant Breeding International, Cambridge, England) which is moderately resistant to powdery mildew and very susceptible to leaf blotch with N.I.A.B. disease resistance ratings for powdery mildew 7; *Septoria tritici* 3; *Septoria nodorum* 4 (Anon, 1994b).

The third variety, cv. Mercia, is a hard endosperm texture, high yielding, red-grained bread wheat bred in England by Plant Breeding International. This cultivar is moderately susceptible to powdery mildew disease caused by *Erysiphe graminis* and moderately susceptible to glume and leaf blotch caused by *Septoria nodorum* and *Septoria tritici.* It has N.I.A.B. disease resistance ratings for powdery mildew 5; *Septoria tritici* 5; *Septoria nodorum* 6 (Anon, 1994b).

2.0.2 Barley

For the purposes of this research the cv. Pastorale (Secobra, France; UK agent; Nickersons Seeds, Rothwell, Lincolnshire) was used. It is a two row winter

cultivar used for animal feed. Although high yielding it is very susceptible to powdery mildew with a N.I.A.B. disease resistance rating of 3 (Anon, 1994b).

2.1 Field Studies

2.1.1 Overall Aims

Four field experiments were conducted over two years. There were three aims to the field experiments. The first aim was to challenge naturally occurring epidemics in cereal crops with fertiliser at different times in the growing season and record any changes in disease severity. The second aim was to identify any differences in disease severity between foliar applied fertiliser and equivalent dressings of solid fertiliser. In the second year experiment, varietal interaction with fertiliser practice was also examined.

2.1.2 Equipment and Techniques

Each field experiment differed in layout, sampling and general husbandry according to the location. In all cases the crops were established after ploughing. The trial plots were marked out using fibreglass canes. Treatments were applied at specific growth stages defined according to the scale of Zadoks, Chang and Konzak (1974). Solid fertilisers were applied by hand broadcasting. Foliar fertilisers were applied using an Oxford precision plot sprayer (M.D.M. Engineering, Southampton, England) using either Chafer green hollow cone (Chafer, England) or Lurmark 02F110 flat fan medium droplet jets (Lurmark, Cambridge, England).

Disease severity was measured using visual assessment techniques. The disease assessor was equipped with comparative charts of foliar disease (Anon, 1976). These were used to provide a reference when the assessor estimated the leaf area affected by a particular disease. The charts represent leaves with specific percentage areas affected. The operator has to interpolate between diagrams to

provide accurate assessments for individual leaves. . Parker *et al.* (1992) suggested that observers are often inaccurate, inconsistent and imprecise in making visual estimates of disease severity. A particular problem identified by these workers was a lack of consistency between assessments made at different times. To alleviate this problem observers used Distrain™ (The Agricultural Research Service of the U.S.D.A, Beltsville, U.S.A.), a computer based training package, to improve the accuracy and consistency of the assessments.

In the first year disease severity scores were recorded on field data sheets. This was time consuming and laborious. In the second year a data logger was used.

2.1.3 Locations

All field experiments were conducted in the vicinity of Harper Adams Agricultural College (H.A.A.C.), Newport, Shropshire, England. The experiments using urea, one on barley and one on wheat, were located in Birds Nest Field at H.A.A.C. Two experiments were conducted using potassium chloride on wheat. The first year experiment involving potassium chloride was conducted in Tank field, Green Farm, Burnhill, Patsall, Shropshire. The second year field experiment was conducted in Large Marsh Field at H.A.A.C.

2.2 Laboratory and Glasshouse Studies

2.2.1 Growth of Plants

Initial attempts were made to grow plant material for experimental purposes in the glasshouse facilities at Harper Adams Agricultural College. Both wheat and barley were grown. The untreated seed was graded for size by passing it over a 4mm top and 3mm bottom sieve and surface sterilised by immersion in 5% sodium hypochlorite solution for five minutes to control seed borne pathogens and washed

before sowing approximately 12mm deep in pots containing John Innes No.3 compost.

This system had two drawbacks. The individual seed germinated unevenly producing batches of plants with uneven development and emerging seedlings were quickly infected by powdery mildew resulting in death. To overcome the unevenness of the plants a new technique was tried. Untreated seed was surface sterilised by immersion in 5% available chlorine solution for five minutes followed by washing five times in sterile de-ionised water. The seed was then placed between layers of paper towels in a beaker and sealed in a plastic bag and incubated at 20°C for approximately 72 hours. Germinated seed was selected for even development and sown approximately 12mm deep in pots containing a specially prepared compost detailed below. This produced much more even batches of plants and resulted in a much lower discard rate when plants were selected for uniformity at the commencement of an experiment and better use of resources. The plants still suffered from infection by mildew. At the suggestion of supervisors, temperature manipulation was tried. Temperature reduction slowed epidemics but merely delayed the destruction. It was considered that no pre inoculation mildew infection could be tolerated for two reasons. Firstly, the response of diseases to fertiliser application may have been dependent upon the development of the pathogen at the time of application, which was unknown in naturally infected plants. Secondly the disruptive effect of mildew, *E. graminis*, on nitrogen metabolism (Sadler and Scott, 1974) may have altered the plant's response to fertiliser treatments. The use of fungicides was precluded due to possible effects on plant metabolism, residue toxicity to inoculated pathogens and interactions between the fungicide residues and the applied fertiliser. As a result mechanical control was tried and a spore free propagator was built.

2.2.2 Construction of a "Spore Free Propagator"

A spore free propagator was built to provide mildew free plants. The basic requirements were for it to be as large as possible and to accommodate wheat plants to maturity if required. To this end three boxes 1450mm wide, 1850mm long and 1250mm high were constructed from 22mm softwood timber frames with sheets of acrylic sheeting (Multiglass™) fastened on with wooden battens nailed to the frame. The sheeting had a light transference of 95.5%. Plywood triangles were used at the corners to provide additional strength (Fig 1). Silicone-rubber based sealant was applied to all plastic-to-wood and wood-to-wood contact edges as a further sealing measure. Removable fronts were held on by bolts, with a strip of foam rubber glued to the back edge of the door frame to ensure a good seal. The floor was constructed from 13mm softwood shuttering grade plywood covered with polythene sheeting and capillary matting. All wood was polyurethane varnished to prevent rot (Plate 1).

Watering was via a 20mm internal diameter pipe passed through the back of the unit to the centre of the floor. Water was poured into the pipe with a funnel and then percolated to the plants through the capillary matting. The tube was sealed between watering, with a cork. Ventilation was provided by a Macklow Centrifugal fan model GG 5 3/4" supplied by Brice Baker (U.K). This fan has a 1/12 h.p. motor powered by a 13 amp electrical power supply. The fan delivered an estimated 200 c.f.m., filtered through a fine particle filter supplied by Thornpark, giving an estimated 1.83 air changes per minute (Plate 2). The air was conducted to each unit via a plastic duct which supplied one lateral pipe per unit. Exit ventilation was provided by a slotted adjustable vent backed by muslin to prevent insect entry. By using a positive pressure ventilation system of the type described, spores could not enter the units during operation and a constant ventilation was achieved at all times. The unit provided a good system for the production of pest and disease free plants as far as was practically possible without recourse to agrochemicals. The "spore free propagator" was used for the production of plant material and initial trials were

successful with no mildew infection in any cabinet which was not opened. Opening cabinets to change the record sheets on a thermohydrograph allowed spores to enter the cabinets and caused a breakdown in disease exclusion. To eliminate this problem it was decided to install electronic probes as part of an improvement programme in the college glasshouse which housed the propagator. Subsequently no disease breakdowns occurred. In the winter, two sodium lamps were suspended above each cabinet to provide eight hours per day of supplementary light.

High summer temperature in the glasshouse (often in excess of 30°C) restricted plant production as the plants grown under such conditions grew quickly producing large flaccid leaves and thin stems. In consequence lodging was very severe, resulting in the rejection of whole batches of plants. It was therefore decided to provide a supply of external air by installing a 15cm internal diameter pipe between the filter unit and the glasshouse wall (Plate 3). This reduced maximal temperatures by up to 10°C resulting in slower growing plants with smaller leaves and shorter, stronger stems which reduced the occurrence of lodging.

2.2.3 Preparation of Potting Compost

Unsuitable potting compost was a major problem encountered in the early stages of the project. The standard potting compost in use at the college at that time was John Innes No.3. Plants grown in this medium grew very quickly and, if vernalised, tillered profusely. The rapidly growing plants were very prone to lodging especially if grown in warm conditions which further exacerbated the problem. Analysis of the compost revealed nutrient levels for each of the nutrients nitrogen, phosphorous (as P_2O_5) and potassium (as K_2O) to be in excess of 1000 ppm. This was much higher than most field soils. Analysis of other commercial composts including seed composts revealed similarly high nutrient contents.

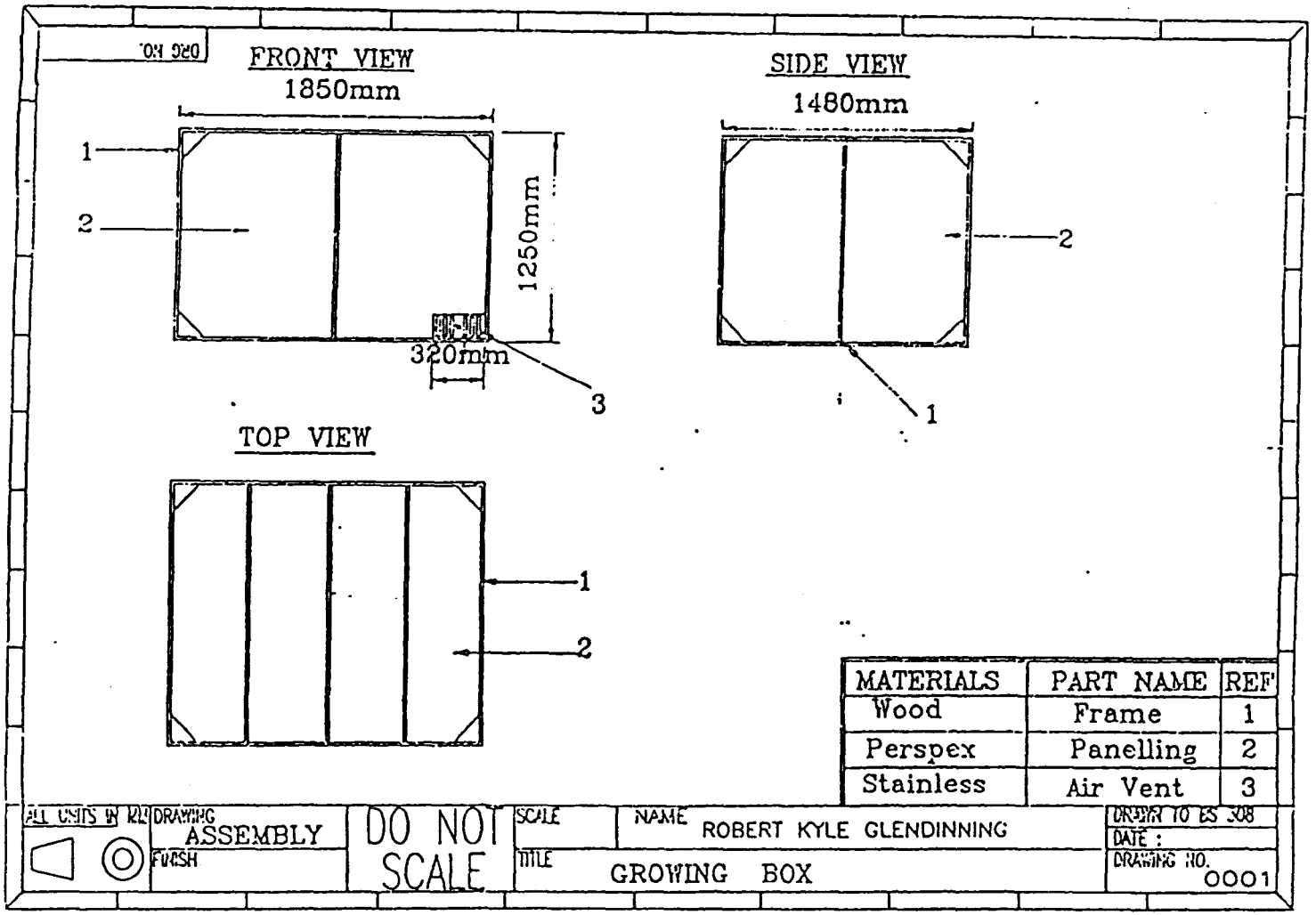


Figure 1. A diagram illustrating the structure of one cabinet forming the spore free propagator (not to scale).(courtesy R.K. Glendinning)

Plate 1. The spore free propagator *in situ*.

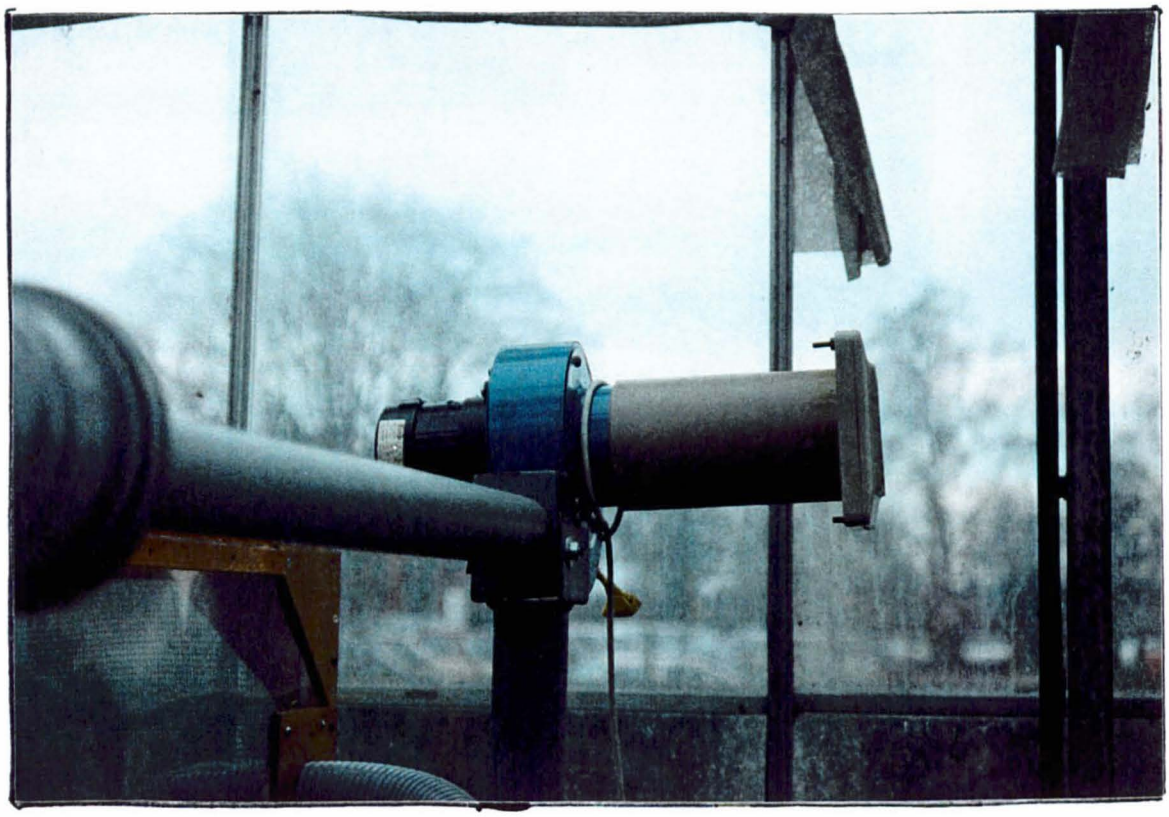


Plate 2. A close view of the centrifugal fan, fitted with filter unit, used to ventilate the spore free propagator.

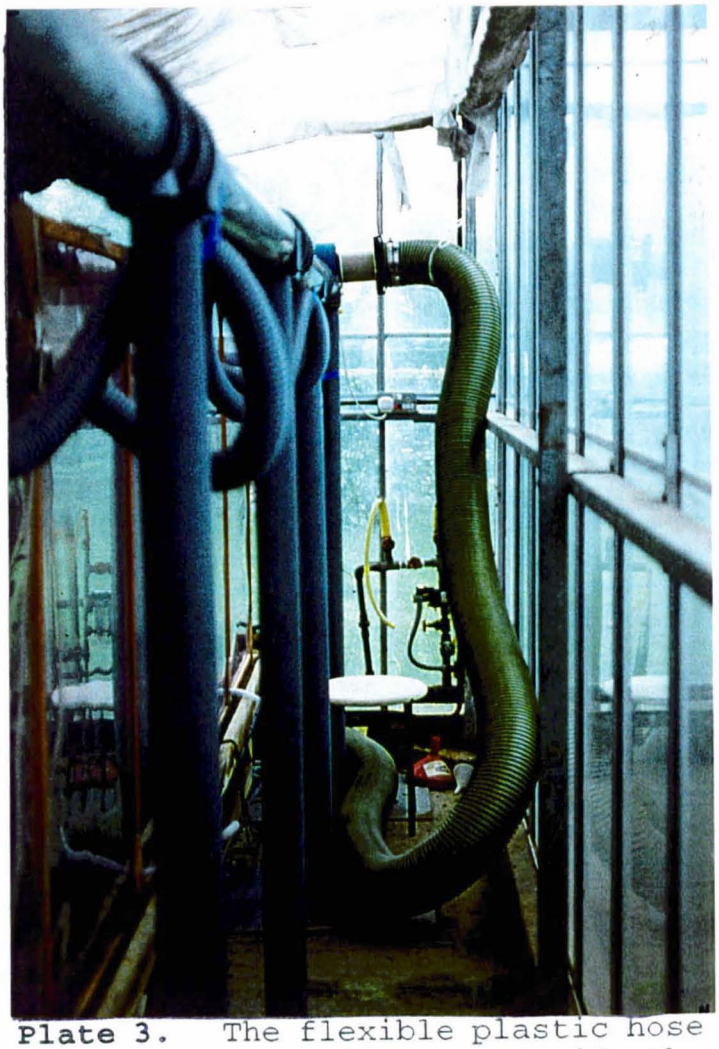


Plate 3. The flexible plastic hose used to conduct air from outside the glasshouse to the filter unit of the spore free propagator.

It was decided to make a special purpose compost very low in potassium. Trial, one litre, mixes were made using a 3:1 peat-sand mix with added fertilisers. Analysis indicated that one hundred grammes of potassium nitrate per cubic metre was satisfactory giving a typical analysis of 20-60 ppm extractable K_2O on a dry matter basis. The average analysis of 40 ppm is in the range within which the Agricutural Development and Advisory Service indicates wheat plants will give yield responses to fertiliser applications (Anon, 1994a). The formula is shown below.

Fertilisers Added to One Cubic Metre of 3:1 Peat:Sand Mix

	kg/m^3
Ammonium Nitrate	0.20
Potassium Nitrate	0.10
Triple Super-Phosphate	1.00
Ground Limestone	2.25
Ground Magnesium Limestone	2.25
Fritted Trace Elements	0.40

2.3.0 Production of Inoculum

2.3.1 Septoria spp.

Septoria was cultured on a modified Czapek-Dox V8 medium as described by Cooke and Jones (1970) details of which are given below.

Czapek Dox V-8 Agar Medium

Campbell's V-8 Juice ... ... 200 ml

Oxoid Czapek Dox (Modified) Agar ... ... 45.4 g

Calcium Carbonate ... ... 3.0 g

Oxoid No.3 Agar ... ... 10.0 g

De-ionised Water ... ... 800 ml

This medium was used for growing all cultures of *Septoria* either at full or half concentration. To produce conidia the full strength medium was used. The plates were inoculated with cirri and incubated as below.

	S. tritici	*S.nodorum*
Initial incubation period in the dark	10 days	2 days
Second incubation period under continuous NUV at 20°C	20 days	16 days

Spore suspensions were prepared by removing all cirri with a scalpel and placing them in sterile distilled water. A suspension was obtained by using a Salvesen homogenising machine for one minute. The suspension was then filtered through two layers of fine muslin. Spore production was poor especially by *S. tritici.* It was later found that the near ultra violet light source was too weak for satisfactory culture of these pathogens (Herbert, *pers. com*).

2.1.2 *Erysiphe graminis*

E. graminis was cultured on stock pots. Approximately 20 pre-germinated seeds were sown 12 mm deep in 75 mm diameter plastic plant pots filled with the special purpose mix used for experiments and detailed above (Plate 4). The pots were placed in a glasshouse bench in a bay kept at a minimum temperature of 15°C by day and 5°C by night. High humidity was maintained by regular damping of the benches. At the two leaf stage the stock pots were inoculated by shaking infected plants over the stock pots to ensure heavy infection.

Plate 4. A stock pot of wheat cv. Apollo heavily infected with powdery mildew (*Erysiphe graminis*) approximately twenty one days after inoculation.

2.4 Data Processing, Graphs and Statistical Analysis

2.4.1 Spreadsheet

Data storage, manipulation and graph drawing was achieved through the use of the computer spreadsheet Quattro Pro™ (Borland International). To avoid transcription error a routine was developed to transfer data from the spreadsheet to statistical packages via a word processor.

2.4.2 Genstat

Genstat was the computer statistics package of choice for this project. Genstat IV was developed for the personal computer at Rothamsted Experimental Station, Harpenden, UK. Genstat is a computing package for data manipulation and analysis which can be used to analyse experiments with a complex or unorthodox design. A later release, Genstat 5, was introduced and used to perform the model building and multiple linear regression towards the end of the project.

2.4.3 Psion Data Logger

The first year field trials revealed the inadequacy of the data acquisition system based on paper records. The time taken to transfer the data from paper to computer and check it for transcription errors took several months and was considered a very inefficient use of time. The use of a data logger was considered to be the solution. Several systems were considered. The Epson data logger was too bulky for use in the kneeling position and required specially written programs. The commercial data loggers such as the Husky Hunter were too expensive for consideration. Finally the O.A.G. logger program (Oxford Agricultural Group, Banbury, England) run on a PSION™ Organiser II was selected. The hardware was widely available and inexpensive (Plate 5). The organiser was small, light and operable with one hand leaving the other hand free for handling the plants being

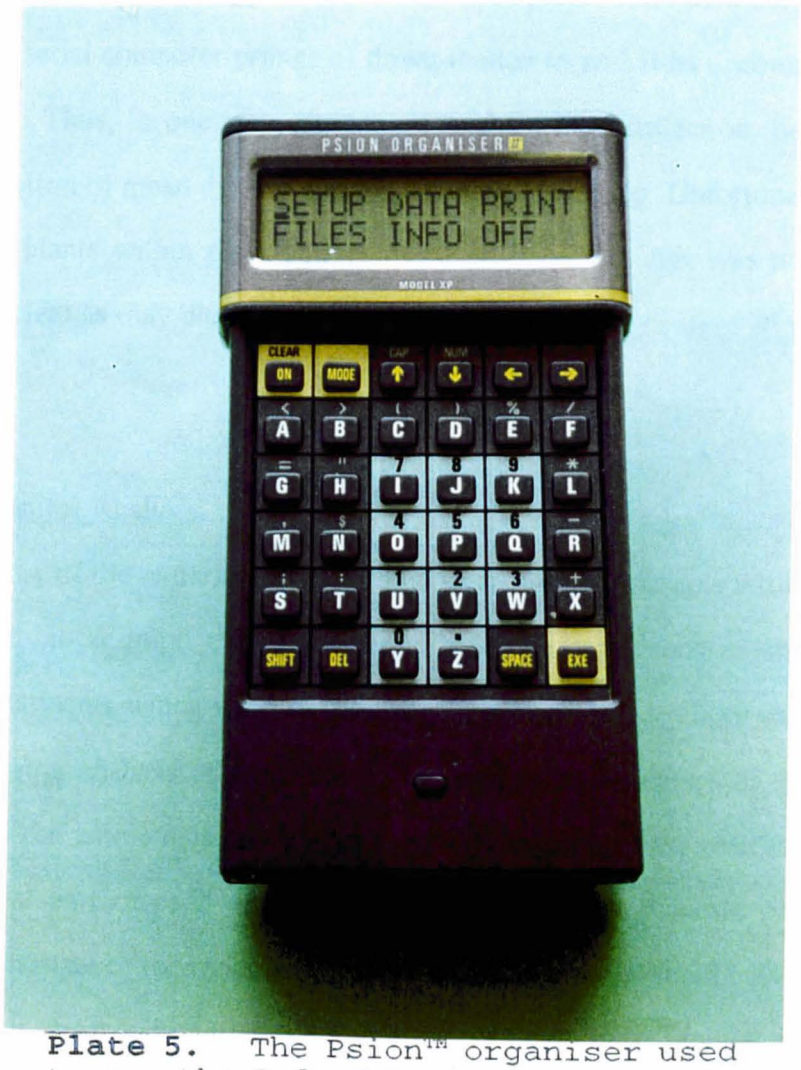


Plate 5. The Psion™ organiser used to run the Oxford Agricultural Group logger program used for recording data in the field.

assessed. The freeing of one hand considerably reduced the time needed to score experiments. In damp conditions the unit could be used sealed in a plastic bag, with a sachet of silica gel, to protect the electronics. The program was flexible and provided ready calculated means for each disease on each plot. The data output was as hard copy on a serial computer printer or down-loaded to an I.B.M™ compatible personal computer. Thus, in one step, the logger enabled data collection, de-randomisation and generation of mean disease scores per plot in one step. Unfortunately scores for individual plants within plots could not be recorded but this was not considered a major problem as only plot means were required.

2.4.4 Statistical Analysis

Most of the experiments described were factorial designs with several factors and levels. In addition each experiment had treatments such as standard practice control treatments which were outside the factorial design. These experiments were analysed using analysis of variance. The residual mean square of the non-factorial analysis of the whole data set was used for the analysis of the restricted data sets of the factorial part of each experiment. Using the larger data set provided a more accurate estimate of the random variation within each experiment. In experiments with four or more quantitative levels orthogonal polynomial analysis was used to test for linear, quadratic and cubic trends in the data. This performed the same function as regression analysis but could be performed more easily as part of the analysis of variance.

In the field experiments the data are presented as percentage leaf area affected. These are mean values derived from twenty individual assessments per plot. According to the central limit theorem the averaging of sub samples will induce a tendency towards normality and therefore satisfy the requirements of the analysis of

variance without transformation. This tendency towards a normal distribution was evaluated and confirmed by producing histograms of the data

Differences between means were examined using Tukey's minimum significant difference test for unplanned comparisons.

2.5. The Precision Pot Sprayer

The precision pot sprayer used in the glasshouse experiments was a custom built apparatus located in the glasshouse at H.A.A.C. It consisted of a mobile spray head incorporating a brass chemical container (Plate 6). The spray head used normal agricultural spray jets and moved along a 1.85m gantry under pneumatic power. The gantry could be raised and lowered to accommodate differences in crop height. The sprayer was located in a walk-in cabinet which was locked during operation (Plate 7). The cabinet was connected to a chemical effluent store and had forced ventilation to the outside of the building. The operations panel was located outside the cabinet.

The application rate was calculated by measuring the quantity of solution used per run and the area of the bench treated. The application rate per square metre was then calculated in litres per hectare.

2.6 Creation Of Solutions Of Known Osmotic Potential.

In latter parts of the experimental programme it became necessary to create control solutions of known osmotic potential to compare with fertiliser solutions. The control substance used was polyethylene glycol which is widely regarded as being biologically inert.

Solution osmotic potential π was calculated according to the formula

$$\pi V = nRT$$

where n moles of substance are contained in a volume V at a thermodynamic temperature T where R is the universal gas constant.

Plate 6. A general view of the precision pot sprayer located at H.A.A.C. closed and ready for use.

Plate 7. An internal view of the precision pot sprayer located at H.A.A.C. showing the spray head, gantry and staging suppports.

Chapter 3. Investigations into the effect of urea on cereal diseases in the field and laboratory.

3.0 Introduction.

The work of Gooding *et al.* (1989) and Peltonen *et al.* (1991) strongly suggested that foliar applied urea would reduce the severity of foliar disease on cereals and therefore might enable fungicide usage to be reduced. Consequently it was decided to evaluate urea both in the laboratory and in the field.

In the experiments of Gooding *et al.* (1989) urea was applied as a foliar spray in addition to normal fertiliser practice. They did not apply equivalent quantities of solid fertiliser to the soil as a control treatment. Therefore it was impossible to determine whether or not the disease control achieved resulted from nutritional effects or some other mechanism.

The objective of the field experiments was to challenge natural epidemics by applying foliar urea in place of a conventional three spray fungicide programme. To account for any possible nutritional effects equivalent applications of solid urea were made to the soil in these experiments.

3.1 Experiment BU1

3.1.1 Objective

To evaluate urea as a disease reducing agent on winter barley when applied as a foliar spray or as a solid granular dressing at different growth stages.

3.1.2 Hypotheses to be tested.

1) Applying part of the total nitrogen as three splits of urea gives better control of foliar diseases than the standard farm practice of two early season split applications with or without three prophylactic applications of fungicides.

2) Applying part of the total nitrogen application as foliar urea gives better control of foliar diseases than applying solid urea prills.

3.1.3 Method

The experiment was of a factorial design with three factors which were the time of application at growth stages (GS) 33 (14.4.92), 47 (15.5.92) and 59 (the treatments to be applied at growth stage 59 were not applied due to above average rainfall which resulted in severe lodging of the crop, consequently analysis of three factor interactions was not possible). Each factor was applied at two statistical levels which were the form of fertiliser, either solid or liquid. The layout of plots was as a four by ten grid with four blocks each containing ten plots. Individual plots measured 1.5 metres wide and approximately 10 metres long. They were separated from other plots by a 0.5 metre path to each side and a one metre discard at each end of the plot. The experimental site was flanked by a guard row to each side one plot wide (Figure 2). The experiment was located in Birds Nest Field at Harper Adams Agricultural College, Newport, Shropshire. The crop followed potatoes and the soil nitrogen index was therefore assumed to be one. All plots received 40 kg/ha of nitrogen as urea prills at growth stage 30. The soil type is a sandy loam.

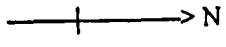
The treatments shown in the table 3.01 were allocated to plots using random number tables. The granular applications, designated solid, consisted of 69g per plot of a high quality urea prill (Kemira U.K., Ince, U.K.). This application, equivalent to 43.48 kg/ha urea or 20 kg/ha nitrogen, was applied to the plots by hand broad casting. Foliar applications, designated liquid, consisted of 220 l/ha of 19.8% w/v urea solution applied using an Oxford precision sprayer using medium cone jets at three bar pressure. This was equivalent to 43.48 kg/ha urea or 20 kg/ha nitrogen. Standard practice, referred to in tables as SP, comprised 207 g solid urea granules (130.43 kg/ha urea or 60 kg/ha nitrogen) at growth stage 33. Standard practice plus fungicides, referred to in tables as SPF, was as standard practice plus applications of 625 ml/ha Punch C™ (flusilazole + MBC) (DuPont U.K., Stevenage, U.K.) at growth stage 33, 625 ml/ha Punch C™ and 1 l/ha Calixin™ (tridemorph) (Bayer,Bury St. Edmunds, U.K.) at growth stage 47 in 220 l/ha water.

The general husbandry of all plots is detailed in Appendix A1.

Figure 2. Plan diagram of the layout of Experiment BU1.

Guard plot	Guard plot	Guard plot	Guard plot	Guard plot	Guard plot
Guard plot	SSL	SLS	LLS	SLL	Guard plot
Guard plot	LSS	SPF	SSS	LLS	Guard plot
Guard plot	SLL	SSS	SSL	SSL	Guard plot
Guard plot	LLL	LLL	SPF	LSS	Guard plot
Guard plot	SLS	SLL	LSL	SPP	Guard plot
Guard plot	SP	SSL	SLS	SLS	Guard plot
Guard plot	SSS	LSL	LLL	SSS	Guard plot
Guard plot	LLS	SP	SPF	LLL	Guard plot
Guard plot	LSL	LLS	LSS	SPF	Guard plot
Guard plot	SPPF	LSS	SLL	LSL	Guard plot
Guard plot	Guard plot	Guard plot	Guard plot	Guard plot	Guard plot

Variety Pastorale



For key to trearments see table 3.01

Table 3.01. Treatments Applied to Experiment BU1.

TREATMENT	GS33	GS47	GS59
DATE	(4.4.92)	(15.5.92)	NA*
1.	Solid	Solid	Solid
2.	Liquid	Solid	Solid
3.	Solid	Liquid	Solid
4.	Solid	Solid	Liquid
5.	Liquid	Liquid	Solid
6.	Solid	Liquid	Liquid
7.	Liquid	Solid	Liquid
8.	Liquid	Liquid	Liquid
9.	SPF (Standard practice + fungicides)		
10.	SP (Standard practice)		

Solid = an application of 43.5 kg/ha solid urea

Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.

SPF (Standard practice + fungicides)= 130.5 kg/ha urea + 625ml/ha PunchC applied at GS33 and 625 ml/ha Punch C™ and 1 l/ha Calixin™ at growth stage 47

SP (Standard practice) = 130.5 kg/ha urea applied at GS33.

* GS 59 treatments not applied due to severe lodging of the crop.

Plots were assessed on 13.4.92 (GS 33), 30.4.92 (GS 34) and 29.5.92 (GS 49) using visual estimation of leaf area affected by comparison of selected leaves with leaf area infection charts. At each assessment the upper two leaves of ten plants were assessed except for the first occasion when only the upper fully expanded leaf was assessed.

Randomised plots were used to satisfy the requirements of analysis of variance. However, sampling within plots was done on a systematic basis for two reasons. Firstly by locating the lines the same distance in from the edge of the plot, which was not considered to have a micro-climate similar to a field crop, edge effect was reduced and also consistent between plots. Secondly, a large number of plants had to be marked and scored and a systematic layout made finding the marked plants much easier and quicker.

At growth stage 32, two lines were marked along each plot approximately 50 cm from the edge using string. Starting 50 cm from the end of the plot, a plant was selected at 90 cm intervals and marked by placing a wire loop below the uppermost fully expanded leaf. In total twenty plants were marked per plot. A plastic label was placed in the soil near each marked plant to aid location. The systematic method was adopted. Plots were not assessed after growth stage 49 due to lodging and very high levels of leaf affected by necrosis. The assessment at growth stage 33 was to check for natural variation in disease levels between plots before treatments were applied.

The data collected at growth stages 33, 34 and 49 was analysed as randomised block designs using analysis of variance. Although the data are given as percentages these are averages for a whole plot and so it was not necessary to transform the data since they tended to normality according to the central limit theorem(see paragraph 2.4.4, p. 37). At growth stage 49 only one plot per block per treatment was assessed to avoid multiple replication of treatments and reduce the

work load needed to complete the experiment. These were selected using random numbers.

Harvesting was carried out on 16.7.92 using a plot combine. Yield was not determined due to severe lodging probably caused by heavier than average rainfall (Plate 8) (Table 3.02). Harvesting with the plot combine produced a heavily contaminated and sprouted sample. A great deal of grain was left in the field and therefore accurate yield determination was impossible.

Table 3.02 . Monthly rainfall and mean daily temperature at Harper Adams Agricultural college during 1992.

Month	Monthly rainfall (mm)		Mean daily temperature (°C)	
March	58.4	(116)	7.1	(131)
April	38.4	(83)	8.6	(113)
May	71.6	(125)	13.2	(122)
June	59.9	(111)	15.2	(110)
July	61.8	(114)	15.8	(101)
August	127.5	(211)	14.8	(96)

Value in parentheses shows the value as a percentage of the 30 year average monthly value 1960-90.

3.1.4 Results

The assessment at growth stage 33, of plots allocated to treatments, before any treatments were applied, revealed no significant differences in the percentage of mildew or net blotch on the upper fully expanded leaf (Table 3.03). By the 30.4.92,

17 days after the application of the treatments at growth stage 33 the application of liquid urea, instead of solid, increased mean mildew infection of the second leaf from 6.3% to 12.6% (P=0.05) (Table 3.04). The application of foliar urea significantly increased the incidence of net blotch (Table 3.04). However the actual level of disease incidence was low. Therefore these results should be treated with extreme caution.

The assessment at GS 49 revealed no significant differences between treatment with respect to powdery mildew or net blotch (Table 3.05). Alternating solid and liquid treatments appeared to give less brown rust infection than applications of all of the fertiliser as either solid or liquid urea. The application of solid urea at growth stage 33 reduced infection of the first leaf by brown rust at growth stage 49 compared to an application of liquid urea (0.7% v 1.2%) irrespective of later fertiliser practice

However the actual severity of the disease was very low and the value of this analysis is not very high. Over the whole experiment fungicides did not appear to have any significant effect on the disease symptoms. This effect was not shown to be significant for any other pathogen.

Plate 8. Severe lodging in Experiment BU1 caused by heavy rain.

Table 3.03. The Assessment of disease severity in Experiment BU1 at growth stage 33 in plots allocated to different treatments before any treatments were applied.

	Powdery mildew	Net blotch
plot group	leaf 1	leaf 1
1	22.5	6.2
2	23.3	10.6
3	24.0	7.3
4	23.6	8.4
5	22.5	10.6
6	20.8	11.5
7	24.4	5.9
9	20.3	5.3
10	21.6	7.9
S.E.M.	1.02	1.23
ANOVA		
treatment	NS	NS
d.f.	26	26
C.V.%	15	46

Each plot group subsequently received the treatments detailed in Table 3.01.

Table 3.04. The effect of treatment on disease severity in Experiment BU1 at growth stage 34.

Treatment	Powdery mildew		Net blotch	
GS33	leaf 1	leaf 2	leaf 1	leaf 2
Solid	0.3	6.3	0.0	0.5
Liquid	0.5	12.6	0.3	1.7
SPF	0.0	3.0	0.0	0.5
SP	0.6	4.4	0.1	0.3
S.E.M.				
Solid v liquid	1.96	1.55	0.09	0.57
SPF&SP means	3.92	3.11	0.18	1.11
M.S.D.				
Solid v Liquid	0.52	6.06	0.03	0.54
SPF v SP	1.05	9.59	0.70	4.41
Other-combinations	0.82	17.25	0.55	3.49
ANOVA				
treatment	NS	*	*	*
d.f.	26	26	26	26
C.V.%	179	75.5	244	243.3

Solid = an application of 43.5 kg/ha solid urea
Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.
SPF= 130.5 kg/ha urea applied at stem extension + 625ml/ha Punch C
SP= 130.5 kg/ha urea applied at stem extension

Table 3.05 The effect of treatment on disease severity in Experiment BU1 at growth stage 49.

Treatment		Powdery mildew		Net blotch		Brown rust	
GS33	GS47	leaf 1	leaf 2	leaf 1	leaf 2	leaf 1	leaf 2
Solid	Solid	1.0	12.3	0.7	2.5	0.1	0.2
Liquid	Solid	1.8	5.6	1.4	1.3	0.3	0.2
Solid'	Liquid	1.1	14.4	0.7	1.9	0.1	0.2
Liquid	Liquid	1.0	9.9	1.0	1.9	0.3	0.6
SPF		0.4	5.6	0.3	1.7	0.2	0.1
SP		1.9	12.8	1.0	3.9	0.1	0.3
S.E.M		0.47	2.91	0.31	1.28	0.07	0.12
M.S.D.		2.17	13.39	1.44	5.88	0.30	0.56
ANOVA							
treatment		NS	NS	NS	NS	NS	NS
GS33		NS	NS	NS	NS	*	NS
GS47		NS	NS	NS	NS	NS	NS
GS32.GS47		NS	NS	NS	NS	NS	*
Split v Solid Solid		NS	NS	NS	NS	NS	NS
SPF v SP		NS	NS	NS	NS	NS	NS
d.f.		15	15	15	15	15	15
C.V.%		78.3	57.7	75.4	117.3	74.3	92.1

Solid= an application of 43.5 kg/ha solid urea at each time.
Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water at each time.
SPF= 130.5 kg/ha urea applied at stem extension + 625 ml/ha Punch C at GS33 and 625 ml/ha Punch C + 1 l/ha Calixin at GS47
SP= 130.5 kg/ha urea applied at stem extension

3.1.5 Discussion

The higher incidence of recorded mildew on the second leaf at growth stage 34 may be explained by reference to the work of Bainbridge (1974) who found that increased infection and sporulation of powdery mildew resulted from increasing the nitrogen nutrition of the host. It was possible that foliar applied nitrogen was absorbed into leaf tissue more quickly and with less loss than soil applied nitrogen resulting in greater growth of the pathogen in the short term. However this effect differs from the observations, on wheat, of Gooding *et al.* (1989) who observed decreases in incidence of mildew when urea was applied.

The higher incidence of brown rust on the flag leaf following the application of foliar urea rather than solid urea at growth stage 33 may have been due to unrecorded differences in canopy structure caused by different growth effects of the early urea applications or due to physiological differences. It appeared that further investigation was required.

The lack of response to fungicides over the experiment was unexpected. It was attributed to the excessive tillering of the crop which produced an extremely dense canopy that subsequently lodged severely (plate 8). This may have inhibited the crops capacity to response to any reduction in disease severity.

3.2 WHEAT UREA EXPERIMENT (WU1).

3.2.1 Objective

To evaluate urea as a disease reducing agent on winter wheat when applied as a foliar spray or as a solid granular dressing at different growth stages under United Kingdom conditions.

3.2.2 Hypotheses to be tested.

1) Applying part of the total nitrogen as splits of urea gives better control of foliar diseases than standard farm practice of two early season split applications with, or without three prophylactic applications of fungicides.

2) Applying part of the total nitrogen application as foliar urea gives better control of foliar diseases than applying solid urea prills.

3.2.3 Method

The experiment was a factorial design with three factors: growth stages (GS)of 32 (17.4.92), 52 (15.5.92), 69 (13.6.92); and two levels: solid and liquid fertiliser. The cultivar of winter wheat was Apollo but due to unforeseen damage to certain plots it was necessary to use one plot per block of cv. Hereward. This was accounted for using covariance analysis. The location, layout and experimental design were as described for experiment BU1. The actual layout is illustrated in Figure 3. Overall husbandry, applied to all plots, is detailed in Appendix A2.

Solid and foliar applications were as described for experiment BU1. Standard farm practice, referred to in tables as SP, was 60 kg/ha nitrogen applied as 130.5 kg/ha solid urea at growth stage 32 in addition to overall husbandry. Standard farm practice plus fungicides, referred to in tables as SPF, was standard farm practice plus applications of 625 ml/ha Punch C™ (Dupont, U.K., Stevenage, England) (Flusilazole + MBC) at growth stage 32, 625 ml/ha Punch C™ and 1 l/ha Corbel™ (B.A.S.F., Hadleigh, UK) (Fenpropimorph) at growth stage 51 and 4 kg/ha Cosmic™ (B.A.S.F.,Hadleigh, UK) (Tridemorph, Carbendazim and Maneb) at growth stage 60. These are summarised in Table 3.05.

The method of sampling was as described for experiment BU1 (paragraph.3.1.3.) Plots were assessed on the dates detailed in Table 3.07. On 16.4.92 the marked leaf was assessed. On 27.4.92 and 7.5.92 the marked leaf and the leaf above, referred to as leaf two and leaf one respectively, were assessed

Figure 3. Plan diagram of the layout of Experiment WU1.

Block A	Block B	Block C	Block D
H SSL	H SLS	H LLS	H SLL
A LSS	A SPF	A SSS	A LLS
A SLL	A SSS	A SSL	A SSL
A LLL	A LLL	A SPF	A LSS
A SLS	A SLL	A LSL	A SP
A SP	A SSL	A SLS	A SLS
A SSS	A LSL	A LLL	A SSS
A LLS	A SP	A SPF	A LLL
A LSL	A LLS	A LSS	A SPF
A SPF	A LSS	A SLL	A LSL

A = cv. Apollo

H = cv. Hereward

Table 3.06. Treatments Applied to Experiment WU1.

TREATMENT	GS32	GS52	GS60
DATE	(17.4.92)	(15.5.92)	(13.6.92)
1.	Solid	Solid	Solid
2.	Liquid	Solid	Solid
3.	Solid	Liquid	Solid
4.	Solid	Solid	Liquid
5.	Liquid	Liquid	Solid
6.	Solid	Liquid	Liquid
7.	Liquid	Solid	Liquid
8.	Liquid	Liquid	Liquid
9.	SPF (Standard practice + fungicides)		
10.	SP (Standard practice)		

Solid = an application of 43.5 kg/ha solid urea

Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.

SPF (Standard practice + fungicides) = 130.5 kg/ha urea applied at GS32 + 625ml/ha Punch C; 625 ml/ha Punch C™ and 1 l/ha Corbel™ at growth stage 51 and 4 kg/ha Cosmic™ at GS60

SP (Standard practice)= 130.5 kg/ha urea applied at GS32

Table 3.07. Dates when Experiment WU1 was assessed.

Plots were assessed on the following dates:

16.4.92 GS 32

27.4.92 GS 33

7.5.92 GS 34

19.5.92 GS 45

11.6.92 GS 59

23.6.92 GS 73

6.7.92 GS 77

14.7.92 GS 85

visually for disease. On 19.5.92 only leaf one was scored due to necrosis of leaf two. After growth stage 34 it was decided not to assess multiple replicates per block due to pressure on time and resources. Therefore only one replicate per block was scored. This was selected using random number tables. At growth stage 39 the selection and marking process was repeated. A waxed paper locking tag was placed around the stem below the second leaf from the top of the plant (Plate 9). Subsequently the uppermost two leaves were scored on each occasion and in some cases the ear as well. Lodging was assessed on 24.6.92 using the method described by Caldicot and Nuttall (1979). The grain was harvested using a Class Dominator plot combine and cleaned using an A/S Rationel Kornservice cleaner. Yields were corrected for mixture and dirt tare. The grain was then passed over a 2 mm sieve on the same machine to determine the percentage screenings.

The data collected were processed as described in general materials and methods and analysed as a randomised block design using analysis of variance. Tukey-Kramer pairwise tests were carried out on the treatment means to compare foliar and solid treatments with standard practice plus fungicides. Although the data are given as percentages these are averages for a whole plot and so it was not necessary to transform the data (see paragraph 2.4.4, p. 37). Meteorological data are given in Table 3.02 (page 46).

Plate 9. The paper tags used to mark individual wheat plants after flag leaf extension. (note the tag has been moved up the stem for photographic purposes.)

3.2.4 Results

The major foliar diseases found in this experiment were *Septoria tritici* and *Erysiphe graminis*. Small quantities of brown rust *Puccinia recondita* were also seen. *S.tritici* and *Fusarium* spp. were the only diseases found to attack the heads on any notable scale. The assessment at growth stage 32 before any treatments were applied to specific plots revealed the presence of powdery mildew *(E. graminis)* and *S. tritici.* The leaf areas affected were low and there was no difference between plots allocated to treatments (Table 3.08).

The percentage leaf area affected by powdery mildew was assessed 10, 20, and 32 days after treatment. Only the first leaf (uppermost fully expanded leaf at the time of treatment) was assessed at the last assessment as the second leaf had died by this time. After ten days there was no difference between the treatments although the covariate was significant reflecting the difference in varietal susceptibility. After 20 days the application of fungicides had reduced the percentage leaf area affected by powdery mildew from 5.8% to 1.7% on leaf 1 when the standard practice plus fungicide treatment was compared with the standard practice treatment (P=0.05). After thirty two days this trend had persisted. Increasing the rate of solid nitrogen fertiliser from 43.5 kg/ha to 130.5 kg/ha increased the percentage leaf area affected by powdery mildew from 6.5% to 12% in the absence of a fungicide (P=0.01). These results are detailed in Table 3.09.

Table 3.08. The assessment of disease severity at growth stage 32 in plots allocated to treatments before treatments were applied to Experiment WU1.

	Powdery mildew	*Septoria tritici*
treatment	leaf 1	leaf 1
1	9.77	3.26
2	7.88	1.65
3	9.14	4.52
4	7.82	4.62
5	9.69	2.76
6	8.83	3.71
7	10.08	2.97
8	8.14	3.61
9	8.51	3.89
10	7.62	3.97
S.E.M.	0.845	0.988
ANOVA		
treatment	NS	NS
d.f.	26	26
C.V.%	19.1	55.9

Each plot group subsequently received the treatments detailed in Table 3.06.

Table 3.09. The percentage area of the leaves of winter wheat cv. Apollo affected by powdery mildew following the application of treatments at growth stage 32 in Experiment WU1.

Treatment	Days post treatment 10 days		20 days		32 days
	leaf 1	leaf 2	leaf 1	leaf 2	leaf 1
Solid	0.9	8.0	5.4	12.8	6.5
Liquid	1.3	6.9	4.5	9.9	4.2
SPF	1.7	6.4	1.7	6.4	2.6
SP	1.0	8.4	5.8	14.0	12.0
S.E.M.					
Solid & Liquid means	0.29	0.72	0.54	1.19	0.54
SPF&SP means & all other comparisons	0.44	1.45	1.08	2.37	0.54
M.S.D.					
Solid v Liquid	1.10	2.74	2.44	4.50	4.64
SP v SPF	1.19	6.47	2.95	2.37	4.64
ANOVA					
treatment	NS	*	*	*	**
Rate N	NS	NS	NS	NS	**
Solid v Liquid	NS	NS	NS	NS	NS
SPF v SP	NS	NS	**	*	*
d.f.	32	32	32	32	8
Covariates	*	***	*	*	*
C.V.%	72.9	38.8	141.4	91.9	45.5

S= an application of 43.5 kg/ha solid urea
L= an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.
SPF= 130.5 kg/ha urea applied at stem extension + 625 ml/ha Punch C
SP= 130.5 kg/ha urea applied at stem extension

The percentage leaf area affected by *Septoria tritici* was assessed at the same time as the powdery mildew. The percentage leaf area affected by *S. tritici* was significantly increased (P=0.05) by foliar applications of urea on both the first and second leaves (Table 3.10) compared with equivalent applications to the soil. This effect did not persist until the second assessment twenty days after treatments were applied.

There was a significant treatment effect (P=0.05) with respect to *S. tritici* severity on leaf one at 20 and 32 days after treatment. However, this appeared to be related to the difference in varietal resistance giving significant covariance rather than any applied treatment. These results are detailed in Table 3.10.

The assessment at growth stage 59, 37 days after the second treatment applications, revealed differences between treatments (Table 3.11). With respect to powdery mildew there was a significant difference in response to the form of urea fertiliser applied at GS 32 (P=0.05) although a clear pattern was not evident in the data. The alternation of solid and foliar applications was apparently conducive to larger areas of the upper leaf being affected by powdery mildew than any other fertiliser strategy (P=0.05). The percentage leaf area affected by *S. tritici* was not affected by any treatment (Table 3.11).

Table 3.10. The percentage area of the leaves of winter wheat cv. Apollo affected by *Septoria tritici* following the application of treatments at growth stage 32 in Experiment WU1.

	Days post treatment				
Treatment	10 days		20 days		32 days
	leaf 1	leaf 2	leaf 1	leaf 2	leaf 1
Solid	0.1	1.5	2.9	10.9	6.8
Liquid	0.3	3.1	2.0	9.73	3.4
SPF	0.1	2.3	6.0	8.9	5.5
SP	0.1	1.2	4.4	7.9	9.3
S.E.M.					
Solid & Liquid means	0.12	0.50	0.71	1.15	1.46
SPF& SP means & all other comparisons	0.06	1.06	2.85	2.31	1.46
M.S.D.					
Solid v Liquid	0.46	1.90	2.6	4.36	6.44
SP v SPF	0.34	3.8	7.7	6.31	6.44
ANOVA					
treatment	*	NS	*	NS	*
Rate N	NS	NS	NS	NS	NS
Solid v Liquid	*	*	NS	NS	NS
SPF v SP	NS	NS	NS	NS	NS
Covariates	NS	**	**	NS	**
d.f.	32	32	32	32	8
C.V.%	141	91	112	94	45

Solid = an application of 43.5 kg/ha solid urea

Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.

SPF = 130.5 kg/ha urea applied at stem extension + 625 ml/ha Punch C

SP = 130.5 kg/ha urea applied at stem extension

Table 3.11. Experiment WU1 The effect of treatment on disease severity at growth stage 59 on 11.6.92.

Treatment		Powdery mildew		Septoria	
GS32	GS52	leaf 1	leaf 2	leaf 1	leaf 2
Solid	Solid	0.7	3.5	1.8	6.7
Liquid	Solid	1.0	3.9	2.7	5.4
Solid	Liquid	1.2	3.5	4.3	7.6
Liquid	Liquid	0.6	2.8	2.8	5.3
S.E.M		0.49	0.48	1.03	1.06
M.S.D.		0.69	2.17	0.07	2.37
ANOVA					
GS32		NS	*	NS	NS
GS47		NS	NS	NS	NS
GS32.GS47		*	NS	NS	NS
Split v SP		**	***	NS	NS
SPF v SP		NS	NS	NS	NS
Covariates		NS	NS	NS	NS
d.f.		14	14	14	14
C.V.%		83	23	33	117

Solid = an application of 43.5 kg/ha solid urea

Liquid = an application of 43.5 kg/ha urea as a foliar spray in 220 l/ha water.

SPF = 130.5 kg/ha urea applied at stem extension + 625 ml/ha Punch C

SP = 130.5 kg/ha urea applied at stem extension

Assessments at growth stage 73 and growth stage 77 revealed that fertiliser type had no effect on the percentage area of the flag (leaf 1) and penultimate (leaf 2) area affected by powdery mildew (Table 3.12). However, split applications produced less powdery mildew than the standard practice with no fungicide treatment on the second leaf at growth stage 73 (P=0.05). *S. tritici* severity was affected by fertiliser type (Table 3.13). Foliar applications of urea at growth stage 57 and growth stage 69 resulted in a greater percentage area of leaf 1 affected by *S. tritici* than solid dressings. Alternated treatments produced intermediate levels of severity. Fertiliser practice had no effect upon ear diseases (Table 3.14).

Yield was increased by the application of split fertiliser dressings rather than standard practice (Table 3.15). The non factorial anova confirmed a significant difference between standard practice plus fungicides and standard practice without fungicides (P=0.05). However, the MSD for all treatments used to compare factorial and non factorial treatments exceeded the difference between standard practice and the factorial treatments indicating no statistically significant yield advantage for any of the factorial treatments. Specific weight was unaffected by treatment (Table 3.15). Thousand grain weight (Table 3.15) was increased by the application of foliar fertiliser at growth stage 69 (P=0.01). Screenings were apparently increased by alternation of solid and liquid fertilisers at growth stage 32 and growth stage 52 (P=0.05) (Table 3.15). Lodging was slightly reduced by the alternation of foliar and solid fertilisers at growth stage 32 and growth stage 52 (P=0.05) (Table 3.13).

Table 3.12. The effect of the form and timing of urea fertiliser application in Experiment WU1 on the percentage area of the flag (leaf 1) and penultimate (leaf 2) leaf of winter wheat (cv. Apollo) affected by *Erysiphe graminis* at growth stage 73 (23.6.92) and 77(6.7.92)

Assessment Date			23.6.92		6.7.92	
Treatment			Leaf 1	Leaf 2	Leaf 1	Leaf 2
GS 32	GS 52	GS 59				
Solid	Solid	Solid	1.2	6.0	3.4	6.8
Liquid	Solid	Solid	1.2	3.9	4.0	8.5
Solid	Liquid	Solid	1.4	3.9	5.6	8.2
Solid	Solid	Liquid	1.8	5.6	4.9	5.5
Liquid	Liquid	Solid	1.7	6.3	2.8	5.5
Solid	Liquid	Liquid	2.2	5.1	5.7	6.7
Liquid	Solid	Liquid	1.5	5.6	3.3	5.4
Liquid	Liquid	Liquid	6.0	6.0	2.2	6.7
SPF			0.8	3.1	1.9	5.4
SP			2.1	7.8	4.5	11.82
S.E.M. main factors			0.23	0.46	1.51	0.76
S.E.M 2 way interactions			0.32	0.23	0.75	1.06
S.E.M 3 way interactions			0.46	0.45	1.06	1.49
MSD			2.2	4.3	7.28	7.28
df			25	25	25	25
CV. %			33	33	42	43
Significance						
GS 32			NS	NS	NS	NS
GS 52			NS	NS	NS	NS
GS 69			NS	NS	NS	NS
GS 32. GS 52			NS	NS	NS	NS
GS 32. GS 69			NS	NS	NS	NS
GS 52. GS 69			NS	NS	NS	NS
GS 32. GS 52. GS69			NS	NS	NS	NS
Solid Solid Solid v SP			*	NS	NS	**
SPF v SP			*	NS	**	NS
Covariate			*	*	**	**

For key to treatments refer to Table 3.06 (page 55).

Table 3.13. The effect of the form and timing of urea fertiliser application in Experiment WU1 on the percentage area of the flag (leaf 1) and penultimate (leaf 2) leaves of winter wheat (cv. Apollo) affected by *Septoria tritici* at growth stages 73 (23.6.92) and 77 (6.7.92).

Assessment Date	23.6.92		6.7.92	
Treatment	Leaf 1	Leaf 2	Leaf 1	Leaf 2
GS 32 GS 52 GS 59				
Solid Solid Solid	3.0	6.2	6.6	20.6
Liquid Solid Solid	3.4	4.7	8.6	13.7
Solid Liquid Solid	2.8	5.5	7.8	16.4
Solid Solid Liquid	4.6	3.5	12.0	17.2
Liquid Liquid Solid	4.7	4.3	14.7	18.84
Solid Liquid Liquid	3.9	5.3	13.1	19.4
Liquid Solid Liquid	4.3	9.5	5.7	22.5
Liquid Liquid Liquid	4.6	4.9	9.1	17.7
SPF	5.4	3.4	5.5	6.5
SP	7.8	7.9	11.6	18.9
S.E.M. main factors	0.42	0.44	8.85	1.97
S.E.M 2 way interactions	0.59	0.63	1.21	2.79
S.E.M 3 way interactions	0.83	0.89	1.71	3.94
MSD	4.39	4.4	8.32	19.2
df	25	25	25	25
CV. %	46	36	34	44
Significance				
GS 32	NS	NS	NS	NS
GS 52	NS	NS	NS	NS
GS 69	NS	NS	NS	NS
GS 32. GS 52	NS	NS	NS	NS
GS 32. GS 69	NS	**	*	NS
GS 52. GS 69	NS	NS	NS	NS
GS 32. GS 52. GS69	NS	*	NS	NS
Solid Solid Solid v SP	NS	NS	NS	NS
SPF v SP	NS	NS	*	*
Covariate	NS	*	NS	*

For key to treatments refer to Table 3.06 (page 55).

Table 3.14. The effect of the form and timing of urea fertiliser application in Experiment WU1 on the percentage incidence of ear disease on winter wheat (cv. Apollo) at growth stages 73 (23.6.92), 77 (6.7.92), and 85 (14.7.92).

Assessment Date	6.7.92	14.7.92	14.7.92
Treatment	*Septoria tritici*	*Septoria tritici*	*Fusarium* spp.
GS 32 GS 52 GS 59			
Solid Solid Solid	4.3	4.8	1.6
Liquid Solid Solid	4.1	3.5	1.7
Solid Liquid Solid	4.1	4.3	2.5
Solid Solid Liquid	4.8	2.8	1.8
Liquid Liquid Solid	5.1	4.2	2.7
Solid Liquid Liquid	4.2	3.2	2.4
Liquid Solid Liquid	4.0	3.8	0.6
Liquid Liquid Liquid	4.8	1.8	1.3
SPF	2.9	4.8	1.9
SP	6.2	3.8	2.7
S.E.M. main factors	0.30	0.08	0.33
S.E.M 2 way interactions	0.43	0.12	0.47
S.E.M 3 way interactions	0.61	0.17	0.66
MSD	2.9	5.0	
df	25	25	25
CV. %	28.3	57	71
Significance			
GS 32	NS	NS	NS
GS 52	NS	NS	NS
GS 69	NS	NS	NS
GS 32. GS 52	NS	NS	NS
GS 32. GS 69	NS	NS	NS
GS 52. GS 69	NS	NS	NS
GS 32. GS 52. GS69	NS	NS	NS
Solid Solid Solid v SP	**	NS	NS
SPF v SP	*	NS	*
Covariate	**	NS	NS

For key to treatments refer to Table 3.06 (page 55).

Table 3.15. The effect of the form and timing of urea fertiliser application in Experiment WU1 on the lodging, yield, specific weight,thousand grain weight and percentage screenings of winter wheat (cv. Apollo).

Treatment	Lodging %	Yield t/ha	Specific weight kg/hl	TGW g	Screenings %
GS 32 GS 52 GS 59					
Solid Solid Solid	62	4.73	69.7	30.68	1.25
Liquid Solid Solid	46	4.85	70.6	31.12	1.43
Solid Liquid Solid	72	4.27	68.9	28.64	1.71
Solid Solid Liquid	60	5.08	70.1	31.93	1.52
Liquid Liquid Solid	58	4.25	69.6	30.86	1.15
Solid Liquid Liquid	43	4.67	71.8	32.26	1.71
Liquid Solid Liquid	52	4.98	70.5	33.21	1.74
Liquid Liquid Liquid	69	5.35	70. 0	32.81	1.29
SPF	51	5.40	71.4	33.05	1.56
SP	69	3.47	70.5	35.53	4.86
S.E.M. main factors	0.08	0.167	0.25	0.353	0.53
S.E.M 2 way interactions	0.19	0.236	0.75	0.499	0.76
S.E.M 3 way interactions	0.17	0.334	1.06	0.706	1.06
MSD	5	2.20	5.2	2.436	7.88
df	25	25	25	25	25
CV. %	31	14.2	3.0	6.5	121.7
Significance					
GS 32	NS	NS	NS	NS	NS
GS 52	NS	NS	NS	NS	NS
GS 69	NS	NS	NS	*	NS
GS 32. GS 52	NS	NS	NS	NS	*
GS 32. GS 69	NS	NS	NS	NS	NS
GS 52. GS 69	NS	NS	NS	NS	NS
GS 32. GS 52. GS69	NS	NS	NS	NS	NS
Solid Solid Solid v SP	NS	**	NS	NS	NS
SPF v SP	NS	*	NS	NS	**
Covariate	NS	*	NS	NS	NS

For key to treatments refer to Table 3.06 (page 55).

3.2.5 Discussion

It would appear that *S. tritici* was generally increased by the substitution of solid by foliar urea. This disagrees with the published findings of Gooding *et al.* (1989), which related to supplemental fertiliser applications, but agrees with the overall findings of Gooding (*pers. com.*) and would appear to exclude foliar urea from a system intended to reduce fungicide inputs in situations where *S. tritici* is expected to be a severe problem.

Possible explanations for this effect were plentiful. Foliar urea may have influenced the pathogen directly acting as a stimulant. Alternatively urea on the leaf surface may have influenced the leaf surface microflora by suppressing competitive saprophytes or encouraging species which produce stimulating factors. The manipulation of the leaf surface microflora by urea application has been shown on apple leaves (Burchill and Cook, 1971).

A more likely reason lies in the physiological activity of urea. Foliar applied urea is widely associated with leaf tissue necrosis or scorch. In this trial a concentration of nitrogen below ten percent was used to minimise the risk of phytotoxic effects (Kettlewell and Juggins, 1992). However the uptake of foliar applied urea may have resulted in symptomless disruption of cell metabolism either by the urea or one of its decomposition products. This could have reduced the phytoalexin response to infection or may have damaged the cells, so facilitating the advance of the pathogen. In contrast the reduction in leaf area affected by powdery mildew was achieved by foliar applications. Jones (1990) indicated that the wax layer of the cereal leaf cuticle is not affected by urea application and hence host recognition is probably not affected. Equally the effect is not likely to be nutritional. Work by Last (1953) clearly indicated that increased nitrogen application (and in consequence increased nitrogen in the tissue) increased the severity of powdery mildew independent of canopy effects. Restricted nitrogen did have an effect in this experiment because split applications resulted in lower leaf areas affected by powdery

mildew than standard practice where all the nitrogenous fertiliser was applied by growth stage 33. As urea can be phytotoxic one cannot discount the possibility of non visible cellular dysfunction. Any dysfunction of the plant cell, even if not lethal, could disrupt the intimate relationship between pathogen and host leading to termination of the biotrophic relationship and death of the pathogen.

Direct toxicity of urea to mildew is a possibility but it is difficult to test this when using fertilisers as the host cannot be excluded from the system and therefore host fertiliser interactions cannot be excluded. One possible mechanism for the mildew reducing action of urea is its conversion to ammonia in the plant. If this ammonia disrupts cellular metabolism the host may be unable to synthesise the essential metabolites necessary to sustain an intimate biotrophic relationship. Equally the ammonia may be toxic to the fungus or block the activity of its exudates which may modify the host cellular function in order to maintain the pathosystem.

3.3 *IN VITRO* EXPERIMENTS.

3.3.1 Introduction.

The use of foliar applied urea to control *S. nodorum* was reported by Peltonen *et al.* (1991). These workers reported that the growth of *S. nodorum* colonies was halted by the addition of 6% w/v urea to the growing media. They indicated that urea was directly toxic to *S. nodorum*. It was considered important to test their findings using British isolates and if possible extend the work to *S. tritici*.

3.3.2 Objective

To determine the effect of urea concentration in the growing media on the growth of *S. nodorum* hyphae.

3.3.3 Method

Czapek-Dox V8 juice was prepared as described in general materials and methods except that one quarter of the water was omitted at mixing. The agar was autoclaved for 15 minutes. The omitted water was sterilised, by autoclaving, and used to dissolve urea to produce solutions which were then filtered through Millipore filters before addition. The concentrations of the urea solutions were such that when added to the agar, after autoclaving, Czapek-Dox agars containing 0,1,2,3,5,6,7 and 8 percent weight / volume urea (BDH, Poole, Dorset, England) were produced. 10 ml of the prepared agar was poured onto each plate using a syringe. 4 mm agar plugs were cut from colonies of *Septoria* belonging to one of two strains using a cork hole borer and then cut transversely just below the visible layer of the colony. The disc of hyphae was then transferred to the prepared plates with a needle using sterile technique. An earlier experiment had indicated the need to produce a thin disc, otherwise the colony did not contact the amended agar and grew in tufts above the plate. Five replicates were used. The plates were incubated at 18°C in the dark.

Measurements of colony diameter were made in two directions, marked onto the plate bases, after four, six and ten days in the second experiment. Analysis of variance and polynomial contrasts were carried out using the Genstat IV package. When analysing the data it was decided to reject data derived from concentrations in excess of three percent because the low or zero colony growth with little variation caused the data to deviate from the condition of normality essential for the analysis of variance.

3.3.4 Results

In experiment SV2 colony growth was apparently stopped by concentrations in excess of 6 percent. Colony growth was only observed on one plate with an added

urea concentration in excess of 5 percent. Treatment means are shown for each assessment in Figures 4, 5 and 6.

The response of the two strains was identical over the first seven days of the experiment. Ten days after inoculation of the plates some difference in colony size was evident between the strains. However, on no occasion was there any interaction between urea and strain. On all assessment dates there was a strong negative linear and quadratic trend in the data (P=0.01). It appeared from the graphs of the data from Experiment SV2 (Figures 4, 5 and 6) that the response to added urea was greatest at low concentrations. The response to added urea declined as the concentration was progressively increased. For completeness means and the full analysis of variance is given in Appendix B1.

Figure 4 Experiment SV2. The effect after four days of increasing the percentage weight/ volume of added urea in the growing media on the colony diameter of *Septoria nodorum* (bar = S.E.M.)

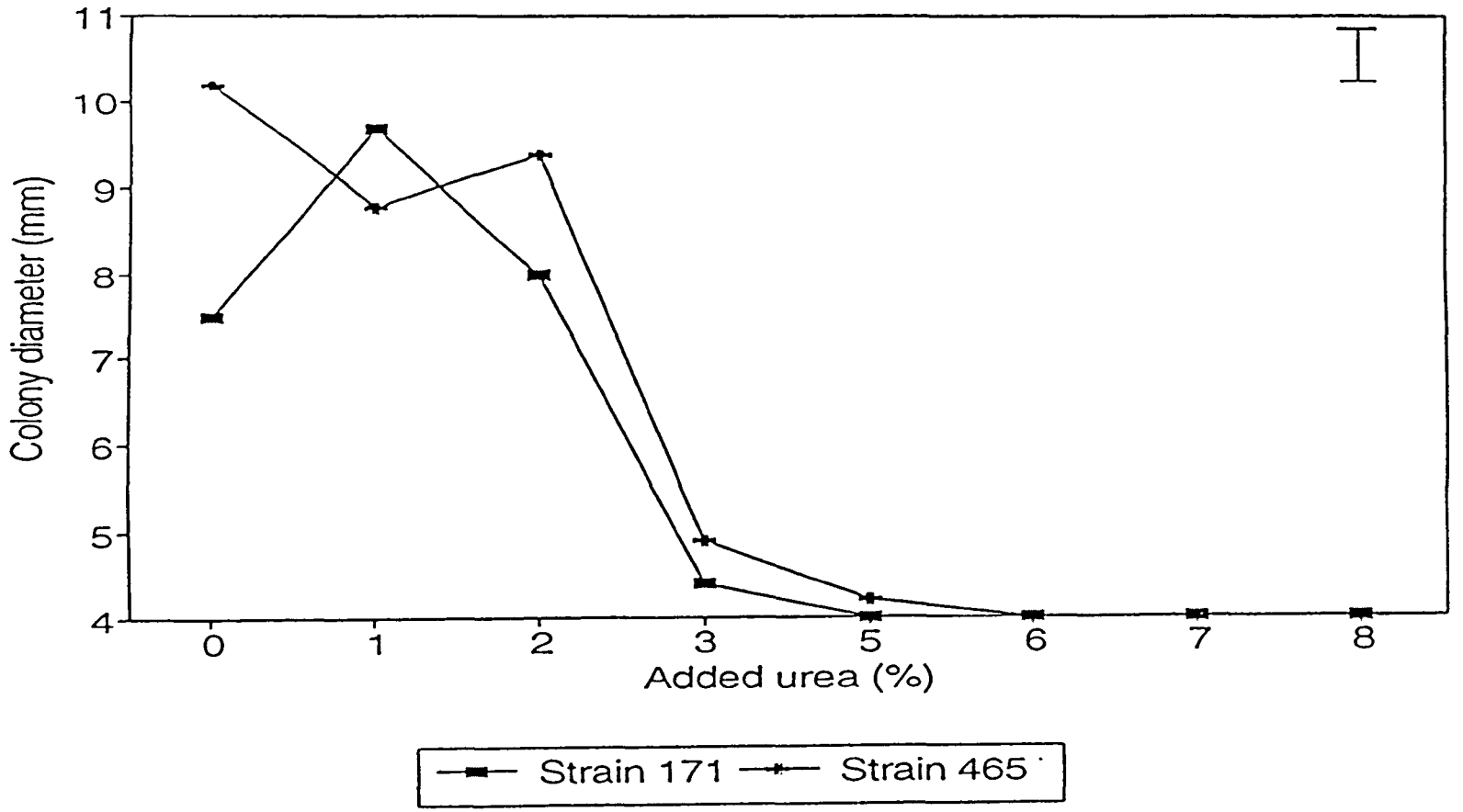


Figure 5 Experiment SV2. The effect after seven days of increasing the percentage weight/volume of added urea in the growing media on the colony diameter of *Septoria nodorum* (bar = S.E.M.)

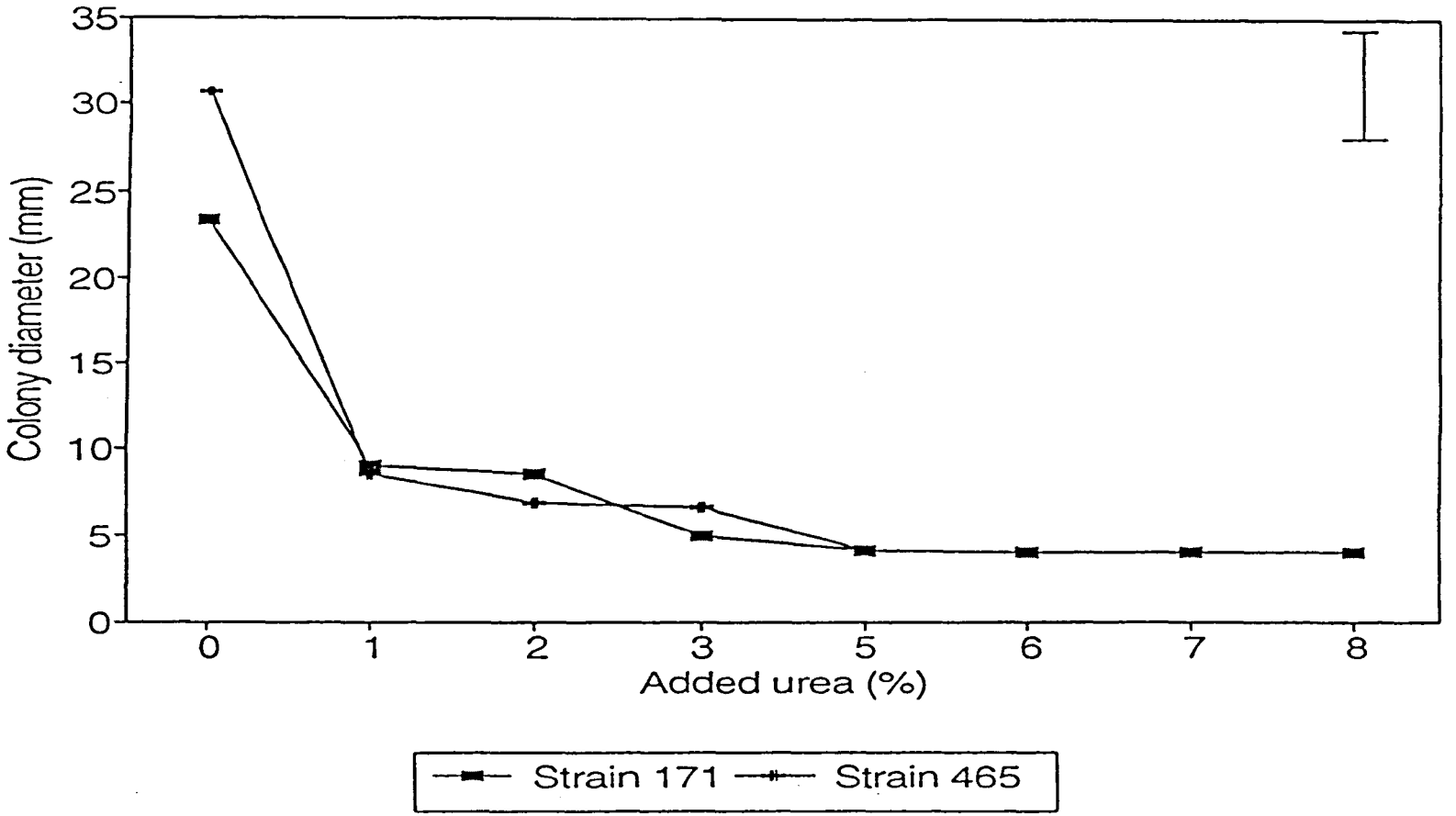
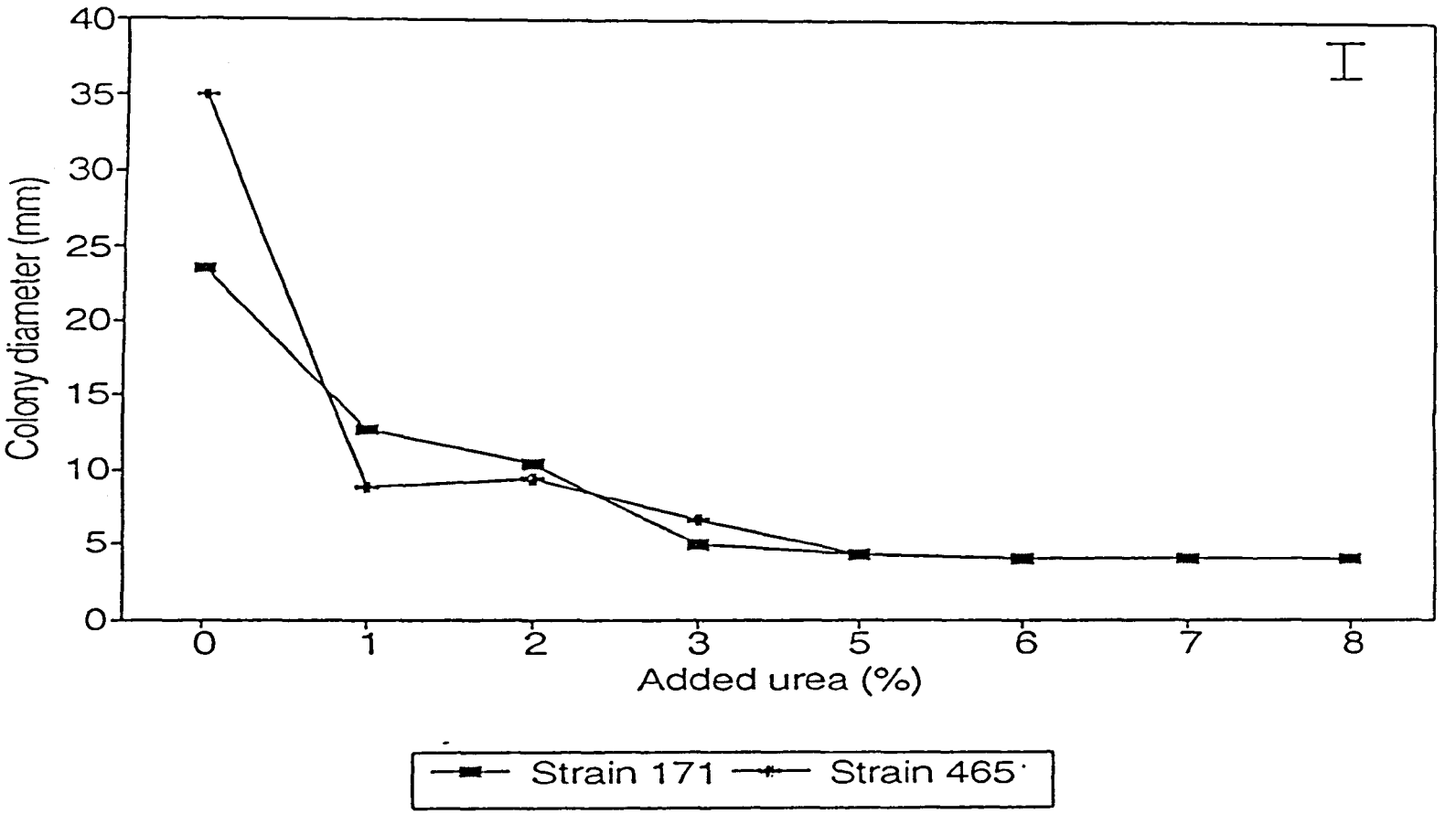


Figure 6 Experiment SV2. The effect after ten days of increasing the percentage weight/volume of urea added to the growing media on the colony diameter of *Septoria nodorum* (bar = S.E.M.)



3.3.5 Discussion

In experiment SV2 increase in colony diameter was apparently retarded by the addition of urea to the growing media. Therefore, it was decided that the results agreed with Peltonen *et al* (1991) that concentrations in excess of six percent added urea completely inhibit the growth of *Septoria nodorum*. It was also concluded that although strains differ in the size of their colonies after identical periods of incubation, the evidence for an interaction between urea and strain was very weak.

3.3.6 OTHER INVESTIGATIONS

Two experiments identical to SV2 were tried using *S. tritici*. These were unsuccessful because colony growth was very slow. In consequence the colonies had become stromatic and ceased to grow before any differences had become apparent. Furthermore, such colony growth as did occur was very uneven making diameter measurements impossible. Reducing the nutrient concentrations in the growing media to half and one quarter strength did not cause the colonies to expand in diameter.

It was decided that a technique should be developed which would enable the effects of foliar fertilisers to be evaluated on leaf sections. Experiments such as the one above were restricted in scope because they could only be used to evaluate the effects of fertilisers on necrotrophic pathogens such as *Septoria*. The agar media techniques were simple but provided no allowance for the dilution effect of plant sap, fertiliser effect on the cuticle of the leaf, the metabolic conversion of the fertiliser in the leaf or possible phytoalexin elicitation. Therefore attempts were made to develop a technique for the evaluation of fertilisers using leaf segments, placed on agar or germination paper and kept alive using cytokinin like compounds. The investigation has so far had limited success as the commonly recommended medium is benzimidazole. This is an MBC generator in the leaf. MBC is a systemic fungicide. It is was therefore considered possible that this might interact with the

urea and influence the results. Kinetin was evaluated as a substitute but as this has less fungistatic activity saprophytic leaf fungi have become a serious problem with this technique.

Development of this technique continued because this technique has immense potential to give greater control over environmental conditions as well as reduce the number of plants needed to screen potential disease controlling fertilisers and evaluate possible variables such as cultivar and fertiliser interactions. However in the short term it was decided to resume work on growing plants until the *in vitro* techniques were perfected. Subsequently a technique was developed where leaf segments were grown in a lighted incubator on a perspex sheet. The basal end of the leaf was inserted through a slot cut in the perspex into a bottle of kinetin solution. In trials it was found that leaves would remain green for up to one week. It was found that 10 ppm kinetin solution maintained the leaves in a visually better state. Unfortunately time did not permit this apparatus to be used in experimental work.

3.3.6 Overall Discussion.

The experiments supported previous work and confirmed that foliar applied urea could reduce the leaf area of cereals affected by *E. graminis*. However the reductions were small, in line with the findings of other workers (Gooding *et al*, 1989). The low level of mildew control coupled with only small effects, or increases, in *Septoria* caused by the application of foliar urea indicated that this line of investigation was unlikely to yield an effective disease control method and it was decided to discontinue the investigation and concentrate on the effects of potassium chloride on cereal diseases.

The different responses of *E. graminis* and *S. tritici* to urea indicated that the fertiliser acted differently on the two pathogens.

The different response of *S. tritici* in experiments where the foliar urea is applied as a supplement compared with the experiment detailed here suggests that the

control reported by Gooding *et al.* (1989) was due, at least in part, to a nutritional effect. The laboratory experiments above indicate that urea is directly toxic to *S. nodorum* and it may also be toxic to *S. tritici* although this was not satisfactorily tested for technical reasons. However in the plant, foliar urea seems to aid pathogen progress compared to soil dressings. This serves to emphasise how *in vitro* screening has limited use in predicting the value of fertilisers as disease control agents.

Chapter 4 - Field Experiments Investigating the Effect of Potassium Chloride On Foliar Diseases of Winter Wheat

4.0. Introduction

The experiments detailed in the literature review indicated that potassium chloride might reduce cereal disease when applied as a foliar spray in the field. This was supported by the work of Kettlewell *et al.* (1990). However the control in these experiments was at low disease severity and only related to applications made late in the season after stem extension. It was therefore decided that a field experiment would be justified to challenge natural epidemics in the field both early in the season and late in the season to evaluate potassium chloride as a disease control agent.

4.1. Field Experiment WKCL1 - 1992

4.1.1 Objective

To evaluate potassium chloride as a disease reducing agent on winter wheat when applied as a foliar spray or as a solid granular dressing at different growth stages under United Kingdom conditions.

4.1.2 Hypotheses to be Tested

1) Applying the total potassium application as three splits of potassium chloride gives better control of foliar diseases than standard farm practice of one early season application with or without three prophylactic applications of fungicides.

2) Applying part of the total potassium chloride application as foliar potassium chloride gives better control of foliar diseases than applying solid potassium chloride prills.

4.1.3 Materials and Methods

The experiment was located in a field adjacent to Patsall Park, Shropshire on a light sandy loam soil. The soil potassium index was 1 (mean 46 mg/l). The cultivar used was Mercia.

The experiment was of a factorial design with 3 factors which were growth stages (GS): growth stage 32 (17.4.92), growth stage 52 (15.5.92) and growth stage 60 (13.6.92) each at 2 levels: solid and liquid. The layout of plots was as a two by twenty grid with four blocks each containing ten plots. Individual plots measured 4 metres wide and approximately 10 metres long. The trial was flanked by a guard row to each side one plot wide (Figure 7).

The experimental treatments were as for Experiment WU1 except that the fertiliser used was potassium chloride (GPR grade, B.D.H., Poole, Dorset, UK). Each level comprised 33.3 kg/ha potassium chloride applied as a solid powder (133 g/plot) or 220 l/ha of 15.1% w/v solution applied as a foliar spray. Standard farm practice, referred to in the tables as SP, comprised 100 kg/ha (399 g/plot) potassium chloride powder applied at growth stage 32. Standard farm practice plus fungicides, referred to in the tables as SPF, was as described with applications of 625 ml/ha of Punch C™ (DuPont, UK, Stevenage, England), a formulated mixture of flusilazole and MBC, at growth stage 32, 625 ml/ha Punch C™ and 1 l/ha tridemorph as Corbel™ (B.A.S.F, Hadleigh, UK) at growth stage 51 and 4 kg/ha Cosmic™ (B.A.S.F, Hadleigh, UK) a formulated mixture of Tridemorph, Carbendazim and Maneb at growth stage 60. All fungicides were applied in 220 l/ha water. These are summarised in Table 4.01. All general husbandry is detailed in Appendix A3. Sampling considerations were the same as in experiment WU1. In the assessments before growth stage 39 a randomised selection of plants was carried out by blind throwing markers into each of the plots to be assessed.

Table 4.01. Treatments Applied to Experiment WKCL1.

TREATMENT	GS32	GS52	GS60
DATE	(17.4.92)	(15.5.92)	(13.6.92)
1.	Solid	Solid	Solid
2.	Liquid	Solid	Solid
3.	Solid	Liquid	Solid
4.	Solid	Solid	Liquid
5.	Liquid	Liquid	Solid
6.	Solid	Liquid	Liquid
7.	Liquid	Solid	Liquid
8.	Liquid	Liquid	Liquid
9.	SPF (Standard practice + fungicides)		
10.	SP (Standard practice)		

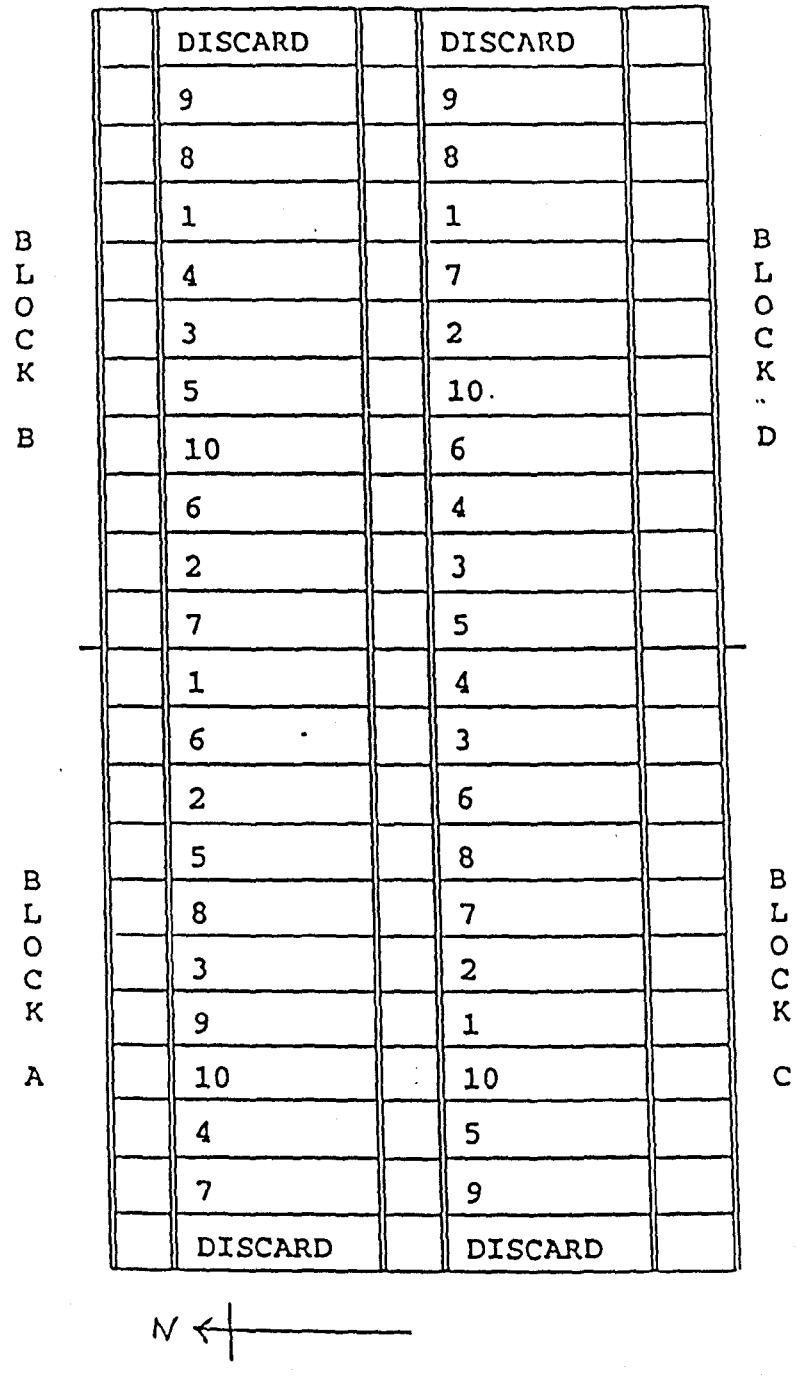
Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

SPF (Standard practice + fungicides) = 100 kg/ha potassium chloride powder applied at GS32 + 625ml/ha Punch C™; 625 ml/ha Punch C™ and 1 l/ha Corbel™ at growth stage 52 and 4 kg/ha Cosmic™ at GS60

SP (Standard practice) = 100 kg/ha potassium chloride powder applied at GS32

Figure 7. Plan diagram of the layout of Experiment WKCL1.



TREATMENT	GS31	GS39	GS51
1.	S	S	S
2.	L	S	S
3.	S	L	S
4.	S	S	L
5.	L	L	S
6.	S	L	L
7.	L	S	L
8.	L	L	L
9.	NORMAL FERTILISER PRACTICE + FUNGICIDE		
10.	NORMAL FERTILISER PRACTICE		

The factorial design of the experiment with treatment times being factors meant that assessing all the plots before the final treatments had been applied at GS60 involved having multiple replicates. After the first assessment multiple replicates were abandoned, due to pressure on time and resources, and only one replicate per treatment per block was scored. This was chosen by random numbers. After the growth stage 51 treatment was applied the systematic system used for Experiment WU1 was used due to the volume of plants to be assessed. The plots were assessed for disease on the dates detailed in Table 4.02. The assessment made before the treatments were applied was to check for innate variation in disease between the plots.

Table 4.02 Dates When Experiment WKCL1 was Assessed

Plots were assessed on the following dates:

20.4.92	GS 32
6.5.92	GS33
13.5.92	GS 34
20.5.92	GS 45
12.6.92	GS59
26.6.92	GS73

On 20.4.92 the uppermost fully expanded leaf was assessed on ten randomly selected plants per plot. On 6.5.92, 13.5.92 and 20.5.92 the penultimate and the uppermost fully expanded leaf, referred to as leaf two and leaf one respectively, were visually assessed for disease. At growth stage 39 a waxed paper locking tag was placed around the stem below the second leaf from the top of the plant on twenty plants per plot. On 12.6.92 and 22.6.92 the flag and penultimate leaves were scored. The results were analysed with ANOVA and Tukey's minimum significant difference test. It was not necessary to transform the data for the reasons described in paragraph 2.4.4 on page 37.

Meteorological data applicable to this experiment are given in Table 3.02 (page 46). Harper Adams Agricultural College is the nearest weather monitoring station to which we had access.

4.1.4 Results

Assessment of the experimental plots before any treatments were applied revealed no significant differences in natural disease incidence (Table 4.03). Powdery mildew was not affected by any treatment one week after treatment on either leaf one or leaf two (Table 4.04). Foliar treatment reduced leaf area affected by powdery mildew from 5.5 to 2.2% compared to the solid treatment when assessed two weeks after application (P=0.01) (Table 4.05) and 5.8 to 1.1% after three weeks (P=0.01) (Table 4.06). There were no apparent differences between treatments with respect to *S. tritici* on leaf one until three weeks post application when foliar treatment reduced severity from 2.7 to 1.1% (Table 4.06).

S. tritici on leaf two was unaffected for one week (Table 4.04). Foliar treatments reduced leaf area affected from 8.4% to 1.7% (P=0.01) after two weeks (Table 4.05) and from 9.9% to 4.5% after three weeks (P=0.01) (Table 4.06).

Assessment at growth stage 59 revealed no significant differences between treatments except for a reduction in the leaf area affected by *S. tritici* on the first leaf due to split applications of potassium chloride (P=0.05) (Table 4.07).

Assessment at growth stage 73 indicated a reduction in powdery mildew from 0.4% to 0.3% on the first leaf (P=0.05) and 3% to 2% on the second leaf (P=0.05) due to the application of foliar in place of solid potassium chloride at growth stage 52 (Table 4.08). The application of foliar instead of solid fertiliser at growth stage 52 reduced *S. tritici* from 27.7% to 18.3% (P=0.05) on the second leaf.

Yield was unaffected by treatment except for an increase in yield of almost 1 t/ha due to the application of fungicide (P=0.001) (Table 4.09). A small increase in thousand grain weight occurred when solid fertiliser was applied at both growth stage 52 and growth stage 69 compared to the application of foliar fertiliser at either or both of these times (P=0.05) (Table 4.09). The applications of fungicides significantly increased specific weight (P=0.05) (Table 4.09).

Table 4.03. Disease severity at growth stage 32 (20.4.92) in plots allocated to treatments in Experiment WKCL1 before any treatments were applied.

treatment	Powdery mildew leaf 1	Septoria leaf 1
1	0.1	1.6
2	0.1	1.8
3	0.2	1.8
4	0.2	1.8
5	0.2	1.1
6	0.3	1.3
7	0.1	2.3
8	0.2	1.7
9	0.3	1.2
10	0.4	1.0
S.E.M.	0.11	0.37
ANOVA		
treatment	NS	NS
d.f.	26	26
C.V.%	113	48

Table 4.04. The effect of treatment on disease severity at growth stage 33 (6.5.92) in Experiment WKCL1.

	Powdery mildew		Septoria	
	leaf 1	leaf 2	leaf 1	leaf 2
Solid	0.3	1.4	0.1	0.5
Liquid	0.4	1.7	0.3	0.8
SPF	0.0	0.8	0.2	0.8
SP	0.2	1.2	0.1	1.1
S.E.M.				
Solid & liquid means	0.24	0.13	0.18	0.18
SPF & SP	0.21	0.49	0.06	1.35
M.S.D.				
Solid & liquid means	0.92	4.75	0.40	0.68
SPF & SP	0.78	1.85	0.23	1.80
ANOVA				
Rate K	NS	NS	NS	NS
Solid v Liquid	NS	NS	NS	NS
SP v SPF	NS	NS	NS	NS
d.f.	33	33	33	33
C.V.%	138	67.8	210	97.4

Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

SPF = 100 kg/ha potassium chloride powder applied at GS32 + 625ml/ha Punch

SP = 100 kg/ha potassium chloride powder applied at GS32

Table 4.05. The effect of treatment on disease severity at growth stage 34 (13.5.92) in Experiment WKCL1.

	Powdery mildew		Septoria	
	leaf 1	leaf 2	leaf 1	leaf 2
Solid	1.8	5.5	1.8	8.4
Liquid	0.8	2.2	0.8	1.7
SPF	1.7	2.6	1.7	5.0
SP-F	1.5	3.7	1.5	5.2
S.E.M.	0.37	0.63	0.37	1.22
M.S.D.	1.6	2.7	0.69	5.3
ANOVA				
Rate K	NS	NS	NS	NS
Solid v liquid	**	**	NS	**
SP v SPF	NS	*	NS	NS
C.V.%	51.9	36.2	51.9	48.5

Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

SP = 100 kg/ha potassium chloride powder applied at GS32 + 625ml/ha Punch

SP = 100 kg/ha potassium chloride powder applied at GS32

Table 4.06. The effect of treatment on disease severity at growth stage 45 (20.5.92) in Experiment WKCL1.

	Powdery mildew		Septoria	
	leaf 1	leaf 2	leaf 1	leaf 2
Solid	0.8	5.8	2.7	9.9
Liquid	0.3	1.1	1.0	4.5
SP+F	0.5	1.4	2.1	6.6
SP-F	1.1	3.4	2.2	5.0
S.E.M.	0.24	0.82	0.54	1.79
M.S.D.	1.86	3.63	2.39	7.93
ANOVA				
treatment	NS	*	NS	*
Rate N	NS	NS	NS	NS
Solid Liquid	NS	**	*	**
SPF v SP	NS	NS	NS	NS
d.f.	9	9	9	9
C.V.%	73.2	56.6	53.1	56.6

Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

SPF = 100 kg/ha potassium chloride powder applied at GS32 + 625ml/ha Punch

SP = 100 kg/ha potassium chloride powder applied at GS32

Table 4.07. The effect of treatment on disease severity at growth stage 59(12.6.92)in Experiment WKCL1.

		Powdery mildew		Septoria	
GS32	GS52	leaf 1	leaf 2	leaf 1	leaf 2
Solid	Solid	0.2	2.2	1.8	3.4
Liquid	Solid	0.3	1.9	2.1	3.3
Solid	Liquid	0.1	2.1	2.5	3.7
Liquid	Liquid	0.2	1.5	1.9	3.0
SP		0.4	0.2	3.9	3.3
SP+F		0.1	0.4	3.4	2.3
S.E.M.		0.08	0.44	0.37	0.41
M.S.D		0.360	2.02	1.86	1.79
ANOVA					
treatment		NS	NS	NS	NS
GS32		NS	NS	NS	NS
GS52		NS	NS	NS	NS
GS32.GS52		NS	NS	NS	NS
Split v SP		NS	NS	*	NS
SPF V SP		NS	NS	NS	NS
d.f.		15	15	15	15
C.V.%		83.6	46.5	31.8	23.6

Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

SPF = 100 kg/ha potassium chloride powder applied at GS32 + 625ml/ha Punch; 625 ml/ha Punch C™ and 1 l/ha Corbel™ at GS 52

SP = 100 kg/ha potassium chloride powder applied at GS32

Table 4.08. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL1 on the percentage area of the flag (leaf 1) and penultimate (leaf 2) leaf of winter wheat (cv. Mercia) affected by *Erysiphe graminis* and *Septoria tritici* on 26.6.92 at GS73.

Treatment			*E. graminis*		*S. tritici*	
			Leaf 1	Leaf 2	Leaf 1	Leaf 2
GS 32	GS 52	GS 60				
Solid	Solid	Solid	0.5	3.2	9.5	34.3
Liquid	Solid	Solid	0.3	2.9	2.3	25.8
Solid	Liquid	Solid	0.2	1.7	2.5	16.5
Solid	Solid	Liquid	0.3	2.6	4.1	21.7
Liquid	Liquid	Solid	0.5	2.9	3.9	23.8
Solid	Liquid	Liquid	0.2	2.5	4.0	17.1
Liquid	Solid	Liquid	0.4	3.9	4.8	29.0
Liquid	Liquid	Liquid	0.1	1.5	4.1	15.8
SPF			0.2	2.6	2.5	10.8
SP			0.5	3.2	3.3	18.3
S.E.M. main factors			0.06	0.27	0.76	2.16
S.E.M 2 way interactions			0.08	0.38	0.07	3.05
S.E.M 3 way interactions			0.11	0.54	1.51	4.31
MSD			3.0	3.0	7.1	21
df			26	26	26	26
CV. %			57	40	74	40
Significance						
GS 32			NS	NS	NS	NS
GS 52			*	*	NS	*
GS 60			NS	NS	NS	NS
GS 32. GS 52			NS	NS	NS	NS
GS 32. GS 60			NS	NS	NS	NS
GS 52. GS 60			NS	NS	NS	NS
GS 32. GS 52. GS60			*	*	NS	NS
Solid Solid Solid v SP			NS	S	NS	NS
SPF v SP			NS	NS	*	*

For details of treatments see Table 4.01

Table 4.09. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL1 on the lodging, yield, specific weight, thousand grain weight and percentage screenings of winter wheat (cv. Mercia).

Treatment			Yield t/ha	Specific weight kg/hl	TGW g	Screenings %
GS 32	GS 52	GS 60				
Solid	Solid	Solid	6.6	74.6	36.15	3.88
Liquid	Solid	Solid	6.2	75.2	36.85	5.21
Solid	Liquid	Solid	6.1	73.9	35.31	7.04
Solid	Solid	Liquid	6.2	74.3	36.16	6.62
Liquid	Liquid	Solid	6.3	73.8	35.69	5.20
Solid	Liquid	Liquid	6.6	74.4	35.80	8.53
Liquid	Solid	Liquid	5.8	74.5	35.14	7.34
Liquid	Liquid	Liquid	6.3	74.0	35.62	5.20
SPF			7.3	76.0	39.09	4.09
SP			6.5	74.1	35.02	8.97
S.E.M. main factors			0.11	0.22	0.383	0.981
S.E.M 2 way interactions			0.15	0.31	0.541	1.412
S.E.M 3 way interactions			0.22	10.40	0.765	1.960
MSD			1.1	2.1	2.43	4.92
df			26	26	26	26
CV. %			7	1	40	62
Significance						
GS 32			NS	NS	NS	NS
GS 52			NS	NS	NS	NS
GS 60			NS	NS	NS	NS
GS 32. GS 60			NS	NS	NS	NS
GS 32. GS 60			NS	NS	NS	NS
GS 52. GS 60			NS	NS	*	NS
GS 32. GS 52. GS60			NS	NS	NS	NS
Solid Solid Solid v SP			NS	NS	NS	NS
SPF v SP			***	**	NS	NS

For details of treatments see Table 4.01

4.1.5 Discussion

The data gathered over the three weeks following the growth stage 32 applications suggested that the form and quantity of potassium chloride had a significant effect upon the severity of foliar diseases on wheat.

A comparison of the solid 33.3 kg/ha potassium chloride treatment and the standard practice 100 kg/ha potassium chloride showed that the quantity of potassium applied had little effect on disease severity early in the growing season at soil index one. However, this conclusion was based only upon data from one year and must therefore be treated with caution. In contrast the form of fertiliser had a great effect upon disease levels.

Foliar application of potassium chloride consistently reduced the leaf area affected by powdery mildew and *Septoria tritici*. Kettlewell *et al.* (1990) reported no control of *E. graminis* or *S. tritici* by foliar applied potassium chloride despite applying up to 37 kg/ha. The apparent difference in results between this experiment and that of Kettlewell *et al.* may be attributed to the difference in the concentrations of the solutions applied. The strongest concentration used by Kettlewell *et al.* was 9.3% w/v in comparison to the 15.1% w/v used in the experiment described above. These workers also reported leaf scorch by foliar fertilisers which was not seen in this experiment. It was thought that the lower volumes of spray applied in this experiment, 220 as compared to 400 l/ha, resulted in the elimination of scorch. It was thought that the low volume spray applied evaporated quickly in the warm weather prevailing at the time of application. This evaporation left inert salt deposits visible on the leaf surface preventing scorch. These deposits were then believed to be slowly re-dissolved by dew resulting in slower uptake by the leaf.

A notable and consistent feature was the seven to fourteen day time lag between spraying foliar potassium chloride and observing its effects. This implied that the foliar spray affected the infection process, the establishment of the

pathogen, or inhibited the young invasive hyphae at the edge of a lesion (in the case of *S. tritici*) and thus prevented lesion expansion.

Possible modes of action included direct activity against spores or hyphae and indirect mechanisms. Direct activity may have been due to spore lysis as a result of changes in the osmotic potential of the leaf surface water or toxicity to the pathogen. Evidence from maize suggests that potassium ions are rapidly taken up over the leaf surface (Chamel, 1988). This may be much faster than translocation from the root. Such a rapid input into a deficient leaf might enhance its response to disease. If the leaves had a sub-optimal content of potassium prior to treatment, this hypothesis might explain the results obtained. It is possible that the soil applied potassium fertiliser was not taken up by the wheat plants although this is unlikely because the soil was moist at this time and showers did occur not long after the application. This might explain the absence of differential responses to the different rates of soil-applied potassium chloride which may not have been due to the lower rate supplying sufficient nutrients but due to a lag phase between uptake and moving those ions to the leaves or even that the soil applied potassium chloride was not taken up due to soil conditions.

Another factor is the chloride input into the leaf from the foliar application which may have been higher than that of the soil application. Unnatural chloride concentrations may have proved fungitoxic in the leaf tissues.

The lack of yield response to disease control might be attributed to serious water logging of the trials site following torrential rain during the grain filling period. This may have prevented the crop exhibiting its full potential although the yields obtained from all treatments were reasonable in relation to site potential. The yield advantage of the fungicide treated plots may have been due to flusilazole controlling stem base disease or the MBC component having a phytotonic effect as has been widely reported. Alternatively the lack of a yield response to the disease control provided by the potassium chloride sprays may

have been due to the foliar application of the salt solution being phytotoxic. Inclement weather delayed the application of the last two fertiliser applications. This may have caused the crop to be deprived of potassium at certain times. This may have restricted responses to disease control which may also have resulted in a loss of potential yield due to nutrient deprivation during grain filling. The latter suggestion would appear to be the most likely in view of the low reserves of available potassium in the soil which meant that a high yielding wheat crop was highly dependent on nutrients being available from fertiliser applications. The standard practice plus fungicide treatment had similar disease control to the foliar fertiliser treated plots but also had adequate fertiliser present from the commencement of the experiment.

4.2.0 Field Experiment WKCL2 - 1993

4.2.1 Objective

To evaluate potassium chloride as a disease reducing agent on winter wheat when applied as a foliar spray or as a solid granular dressing at different growth stages under United Kingdom conditions and to determine the difference in response of two wheat cultivars. The latter objective involved comparing very susceptible and partially resistant cultivars to answer Huber's (1980) suggestion that disease reductions due to nutritional changes were more pronounced in moderately resistant genotypes than very susceptible ones. A comparison of disease severity was also made between the experimental husbandry techniques and conventional practice.

An attempt was made to relate potassium fertiliser practice to the tissue concentration of potassium in the leaf. Conventional practice is to express potassium on a dry matter basis due to diurnal and daily changes in moisture content. However Barraclough (*pers. com.*) suggested calculating uptake on a tissue water and wet matter basis. Following Barraclough and Leigh (1993) it was considered that leaf potassium content on a wet basis is more relevant to the element's biophysical functions within a plant. It was also suggested that the concentration of potassium in tissue water is independent of the supply of other nutrients. These workers were concerned with crop yield. It was logical to extend their hypothesis to disease susceptibility.

4.2.2 Hypotheses to be Tested

1) Applying the total potassium application as three splits of potassium chloride gives better control of foliar diseases than standard farm practice of one early season application with or without three prophylactic applications of fungicides.

2) Applying part of the total potassium chloride application as foliar potassium chloride gives better control of foliar diseases than applying solid potassium chloride prills.

3) Changes in disease severity as a result of fertiliser practice are more pronounced on partially resistant than very susceptible cultivars.

4.2.3 Method

The experiment was located in Large Marsh field at Harper Adams Agricultural College, Shropshire on a light sandy clay loam soil. The soil potassium index was 0 (mean 34 mg/l).

The experiment was a factorial design with three factors which were growth stages: growth stage 31, growth stage 39 and growth stage 59, each with two levels; solid and liquid and one factor, cultivar, with two levels: cv. Apollo and cv. Riband. Each growth stage level comprised 33.3 kg/ha potassium chloride applied as a solid powder (133 g/plot) or as a foliar spray 220 l/ha water foliar spray of 15.14% w/v solution. In addition four treatments were included in the experiment outside the factorial design. These were standard practice, standard practice plus fungicides, split applications of potassium chloride plus a foliar spray of water at each application and split applications of potassium chloride plus fungicide.

Standard farm practice, described as SP in tables, comprised 100 kg/ha (399 g/plot) potassium chloride powder applied at growth stage 31. Normal farm practice plus fungicides, described as SPF in tables, was as described with applications of 300 ml/ha Sanction™ (Dupont, U.S.A) (Flusilazole) and 750 ml/ha Bas 464™ (B.A.S.F, Germany) (Fenpropimorph + Tridemorph) at growth stage 31, 1000 ml/ha Tern™ (Ciba Geigy, Switzerland) (Fenpropidin) and 500 ml/ha Tilt™ 250EC (Ciba Geigy, Switzerland) (Propiconazole) at growth stage 39 and 4 kg/ha Cosmic™ (B.A.S.F, Germany) (Tridemorph, Carbendazim and Maneb) at

growth stage 59. All treatments were applied in 220 l/ha water. Split applications plus a foliar spray of water consisted of an application of 33.3 kg/ha solid potassium chloride at GS 31, 39 and 59 plus a foliar spray of 220 l/ha water. Split applications plus fungicides consisted of an application of 33.3 kg/ha solid potassium chloride at GS 31, 39 and 59 plus fungicides as detailed for standard practice plus fungicides.

Each chemical treatment was applied to two cultivars, Apollo and Riband. The experiment was of a split plot design laid out in three blocks (Figure 8). The split plot design was used in preference to a fully randomised design because the varieties differed in disease susceptibility and it was thought that differences in potential inoculum production would occur leading to interference between plots. Therefore it was decided to isolate each variety within a block. The plots within each block were allocated to one cultivar or the other by random numbers. Within each plot treatments were allocated using random numbers. Individual plots measured 1.5 metres wide and approximately 10 metres long. Each varietal block was flanked by a guard row to each side one plot wide and separated from the other variety at the end of the plot by an overrun. Treatments were applied as detailed for experiment WKCL1. All general husbandry is detailed in Appendix A4.

Sampling considerations were the same as in experiment WU1. Multiple replicates were not scored in this experiment. One replicate per treatment per block was scored. This was chosen by random numbers. The plots were assessed for disease as described for experiment WU1. The dates are detailed in table 4.11.

Table 4.10. Treatments Applied to Experiment WKCL2.

TREATMENT DATE	GS31 (30.4.93)	GS39 (12.6.93)	GS59 (15.7.92)
1.	Solid	Solid	Solid
2.	Liquid	Solid	Solid
3.	Solid	Liquid	Solid
4.	Solid	Solid	Liquid
5.	Liquid	Liquid	Solid
6.	Solid	Liquid	Liquid
7.	Liquid	Solid	Liquid
8.	Liquid	Liquid	Liquid
9.	SPF (Standard practice + fungicides)		
10.	SP (Standard practice)		
11.	Solid + Water	Solid +Water	Solid + Water
12.	Solid +F	Solid+F	Solid+F

Solid = an application of 33.3 kg/ha solid potassium chloride

Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.

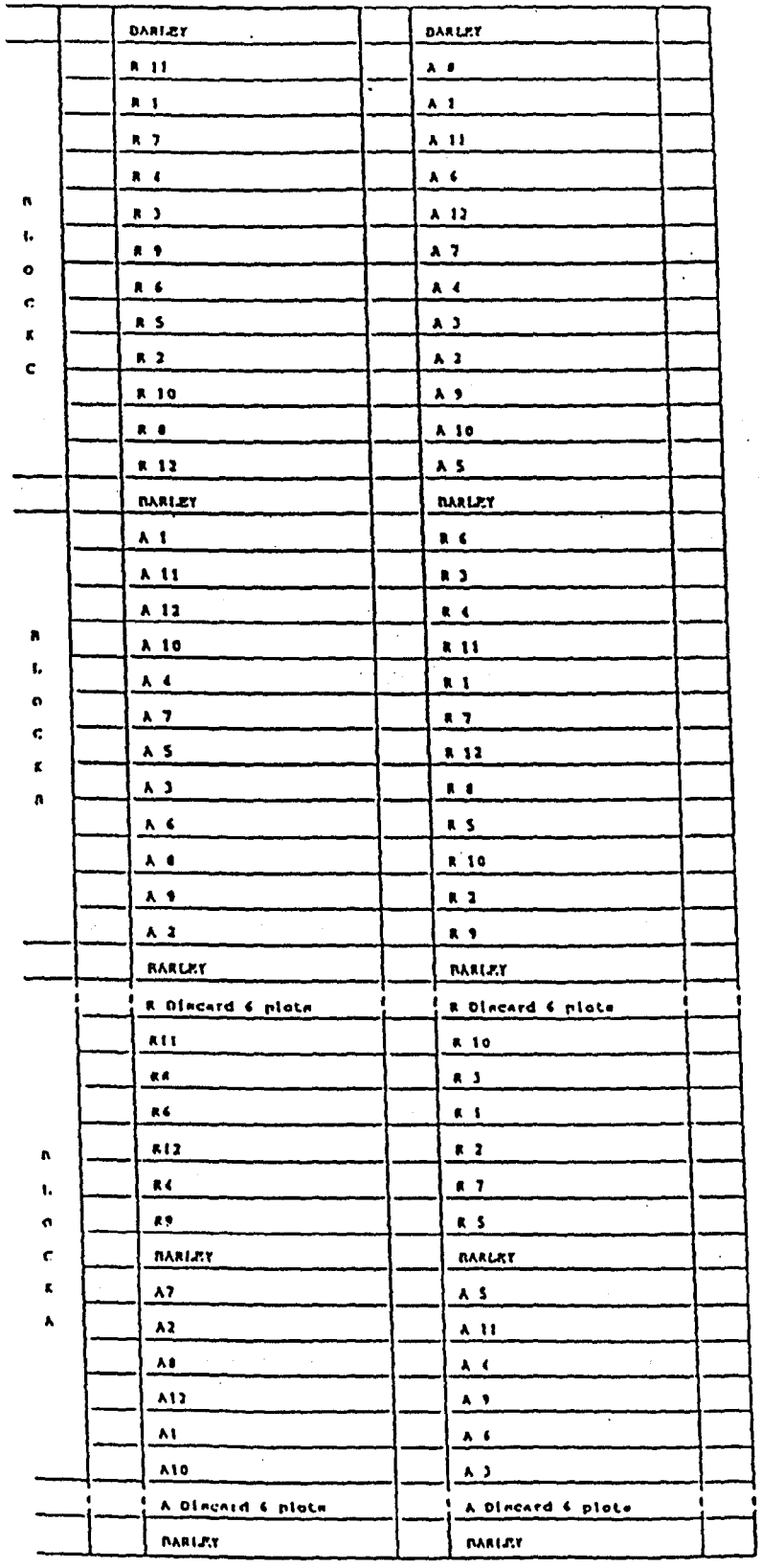
SPF(Standard practice + fungicides) = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml BAS 464 ™; 1000 ml/ha Tern ™ and 500 ml/ha Tilt ™ 250EC at growth stage 39 and 4 kg/ha Cosmic™ at GS59

SP(Standard practice) = 100 kg/ha potassium chloride powder applied at GS32

Solid + Water(Split solid + water) = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.

Solid +F (Split solid + fungicide) = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction™ + 750 ml BAS 464 ™ applied at GS31, 33.3 kg/ha solid potassium chloride + 1000 ml/ha Tern ™ and 500 ml/ha Tilt™ 250EC at GS39 and GS59, 33.3 kg/ha solid potassium chloride + 4 kg/ha Cosmic™ at GS59.

Figure 8. Plan diagram of the layout of Experiment WKCL2.



For key to treatments see table 4.10

Table 4.11 - Dates when Experiment WKCL2 was Assessed

Plots were assessed on the following dates:

29.4.92 GS 31

4.5.92 GS 32

11.5.92 GS32

18.5.92 GS 33

4.8.92 GS 77

On 29.4.92 the uppermost fully expanded leaf was assessed on twenty plants per plot which were selected and marked as described in Experiment WU1. On 6.5.92 and 13.5.92 the marked leaf was scored. The leaf above the marked leaf was scored on 13.5.92 and 20.5.92. This leaf (above the marked leaf) was referred to as leaf one and the marked leaf as leaf two. At growth stage 39 a waxed paper locking tag was placed around the stem below the second leaf from the top of the plant on twenty plants per plot. Subsequently the flag leaf was scored on 4.8.92 by a technician. No disease symptoms were visible on these leaves before the application of treatments at GS 59. This was later than originally planned. On each occasion assessments were made using disease assessment keys (Anon 1976). The data were analysed by analysis of variance and Tukey-Kramer pair-wise tests were carried out on the treatment means. Transformations of the data was not required for the reasons described in paragraph 2.4.4 (p. 37). Due to a high level of leaf death at growth stage 77 it was decided to use only the assessments of live leaves for the analysis of data relating to brown rust (*Puccinia recondita*) and powdery mildew as these biotrophic pathogens cannot live on dead leaves.

The southern 1.5m of each plot was not assessed for disease. The areas between 0.5m and 1.5m from the plot end were destructively sampled to provide tissue samples for later analysis. Samples were taken immediately before and one and four days after treatment application. A sample of twenty five leaves,

equivalent to the marked leaf were detached, sealed in cling film and placed in a cool box. The samples were selected on a systematic pattern to avoid edge effects. The pattern chosen was a five by five grid of approximately equidistant points starting two rows in from the plot edge. The samples were then transported to the laboratory, weighed and then washed for one second in de-ionised water before being dried in an oven at approximately 45°C. The tissue samples were then ground to pass through a 1mm sieve and re-dried before being stored in sealed plastic bags.

Sub-samples weighing 0.4g were analysed for potassium concentration using a flame photometer. The method of preparation was a modified version of the Method 3 dry combustion technique described by A.D.A.S. (Anon, 1986). A 0.4g sample was weighed into a crucible and carbonised by heating to 500°C overnight in a muffle furnace. 2ml of hydrochloric acid (6M) was added to the ash and the crucible was covered with a watch glass to prevent losses due to effervescence. The watch glass was removed and washed. The washings were collected in the crucible. The crucible was then placed on a hot plate and heated until all moisture had evaporated. The residue was heated at 102°C for one hour. 0.2ml of 36 m/m HCl was added and the residue transferred to a 10ml volumetric flask and made up to 10ml. The solution was then filtered through a Whatman No.541 filter paper. The first 1ml of filtrate was rejected and the rest collected. This extract was then analysed using a Fisons Instruments flame photometer. These results were then converted to tissue ion concentrations on a dry matter, fresh matter and tissue water basis. Differences in ion concentration between treatments were then statistically analysed as described for the disease levels. Correlation between tissue ion concentration and leaf area affected by powdery mildew was attempted using regression analysis. For these analyses standard practice plus fungicide treatments were excluded. Samples collected after flag

leaf emergence were not analysed as there were no significant differences in the disease data for the results to be compared with.

An assay for chloride was attempted but no consistent and rapid method was developed with the equipment available at the time. Meteorological data for this experiment are given in Table 4.12

Table 4.12 . Monthly rainfall and mean daily temperature at Harper Adams Agricultural college during 1992.

Month	Monthly rainfall (mm)		Mean daily temperature (°C)	
March	13.4	(38)	6.2	(114)
April	56.9	(122)	9.2	(120)
May	98.1	(171)	11.2	(104)
June	68.9	(128)	15.2	(110)
July	74.1	(136)	12.9	(83)
August	43.8	(73)	14.6	(95)

Value in parentheses shows the value as a percentage of the 30 year average monthly value 1960-90.

4.2.4 Results

Powdery mildew, brown rust and *S. tritici* were the only diseases observed in this experiment. During the early part of the season powdery mildew was the only disease severe enough to be scored on the marked leaf. Traces of *S. tritici* were observed on necrotic lower leaves. There was no significant difference in the severity of powdery mildew on plots allocated to different treatments within varieties on the day before treatment at growth stage 31 (Table 4.13). However, there was a significant difference in the leaf area of each variety affected by powdery mildew (P=0.05). Cv. Apollo had over twice the leaf area affected as cv.

Table 4.13. The percentage area of the upper fully expanded leaf of winter wheat affected by powdery mildew at growth stage 31 in plots allocated to treatments before any treatments were applied to Experiment WKCL2.

Powdery mildew

variety treatment	Apollo	Riband	Mean
1.	9.9	3.9	6.9
2.	10.1	2.6	6.4
9.	11.0	3.6	7.3
10.	8.7	3.8	6.3
11.	9.9	5.6	7.8
12.	9.6	4.6	7.3
Mean	9.9	4.0	

S.E.M.	
application	0.56
variety	0.84
variety * application	1.11
applications within variety	0.79

ANOVA

variety	*
application	NS
variety * application	NS
d.f.	26
C.V.%	
variety	20.9
treatments	19.8

For treatment details see table 4.10

Riband (9.9% v 4.0%). No significant differences were found between treatments within varieties four days after treatment (Table 4.14).

The marked leaf showed significant differences in the leaf area affected by powdery mildew with respect to both variety (P=0.05) and applications (P=0.05) 11 days after treatments were applied (Table 4.15). There was no interaction between variety and applications. Liquid foliar applications resulted in significantly lower mean leaf areas affected than both the solid and standard practice treatments. The level of control provided by a foliar spray of potassium chloride was similar to that provided by standard practice and solid treatments combined with fungicides (Table 4.15). There was no significant difference between the leaf area affected by powdery mildew in the standard practice and solid treatments, indicating that the quantity of fertiliser applied was not a factor affecting disease severity.

Similar patterns were apparent 11 and 18 days after treatment on the leaf above the marked leaf (Tables 4.16 and 4.17). The significance of the difference between applications rose to P=0.001 after 18 days (Table 4.17).

There were no significant differences between the plots allocated to treatments with respect to potassium ion concentration before the application of treatments (Table 4.18). One day after treatments were applied no significant differences were seen between treatments. Four days after treatment application, differences between treatments were apparent on a fresh matter and tissue water basis (P=0.01) (Table 4.20) but not on a dry matter basis (Table 4.20). The foliar treatment was higher than the non-fungicide treated controls.

The elevated potassium concentration created by the foliar treatment was associated with low leaf areas affected by *E. graminis* 11 days after treatment. This may indicate a link between potassium ion concentration following foliar applications of potassium chloride solution and subsequent disease severity.

It was found impossible to get good correlation between leaf area affected by *E. graminis* and the concentration of potassium in the tissues due to a small number of plots and the high number of individual data points which were far from the general trend so increasing variance to a very high level. The removal of a large number of these outlying data from the data to improve correlation was considered to be unsound.

The assessment of the flag and second leaves at growth stage 77 revealed that traces of brown rust, powdery mildew and *S. tritici* were present. There were significant differences between varieties with respect to brown rust and powdery mildew on the flag leaf (P=0.05) (Tables 4.21 and 4.25). On the second leaf varietal influences were noted with respect to *S. tritici* (P=0.001) with cv. Riband being more severely affected (Table 4.24). The treatments applied had no significant effect on the severity of any disease observed on the flag leaf.

On the second leaf the application of fungicides to the standard practice treatment reduced leaf area affected by *S. tritici* from 12.6% to 10.3% (P=0.05). There was also a significant growth stage 31 * variety interaction although no clear pattern was evident (Table 4.24).

Yield was affected by variety (P=0.001). Cv. Riband out-yielded Apollo by approximately one tonne per hectare (Table 4.27). There was a GS39 interaction with treatment (P=0.05) with solid fertiliser giving slightly higher

yields. There were GS31*GS39*GS59 and variety* GS31*GS39*GS59 ineractions but no clear patern was evident and these may be spurious significances. Specific weight was subject to a growth stage 59 * variety interaction but no clear pattern was evident in the data (Table 4.28). Thousand grain weight was increased significantly on both cultivars by the use of fungicides (P=0.05) and this is shown in Table 4.29.

Table 4.14. The effect of treatments applied at GS31 on the percentage area of the marked leaf (leaf 2) affected by powdery mildew in Experiment WLCL2 on 4.5.92.

	Powdery mildew		
treatment	Apollo	Riband	Mean
Solid	10.8	9.2	10.0
Liquid	8.9	8.1	8.5
SPF	10.6	9.2	9.9
SP	9.8	9.9	9.9
Solid+F	10.9	10.8	10.8
Solid+Water	17.0	10.6	13.8
Mean	11.34	9.64	

M.S.D.	
applications	3.99
treatments	7.87
S.E.M.	
application	1.11
variety	2.93
variety * application	3.26
applications within var.	1.57
ANOVA	
variety	*
application	NS
variety * application	NS
d.f.	20
C.V.%	
variety	48.4
treatments	26.0

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 3.33 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 3.33 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.15. The effect of treatments applied at GS31 on the percentage area of the marked leaf (Leaf 2) affected by powdery mildew in Experiment WLCL2 on 11.5.92.

Powdery mildew

treatment	Apollo	Riband	Mean
Solid	24.9	15.9	20.4
Liquid	20.5	7.4	13.9
SPF	21.6	6.1	13.9
SP	30.4	12.8	21.6
Solid+F	20.4	11.2	15.8
Solid+Water	22.5	14.5	18.5
Mean	23.4	11.3	

M.S.D.	
applications	6.03
treatments	9.61
S.E.M.	
application	1.36
variety	1.49
variety * application	2.30
applications within var.	1.99
ANOVA	
variety	*
application	*
variety * application	NS
d.f.	20
C.V.%	
variety	14.8
treatments	19.2

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.16. The effect of treatments applied at GS31 on the percentage area of the upper fully expanded (Leaf 1) leaf affected by powdery mildew in Experiment WKCL2 on 11.5.92.

	Powdery mildew		
treatment	Apollo	Riband	Mean
Solid	5.1	2.7	3.9
Liquid	2.5	2.4	2.5
SPF	3.8	1.7	2.7
SP	4.0	3.4	3.7
Solid+F	2.7	1.9	2.3
Solid+Water	3.7	2.9	3.3
Mean	23.4	11.3	

M.S.D.	
applications	1.61
treatments	2.57
S.E.M.	
application	0.36
variety	0.22
variety * application	0.52
applications within var.	0.51
ANOVA	
variety	*
application	*
variety * application	NS
d.f.	20
C.V.%	
variety	12.1
treatments	29.0

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.17. The effect of treatments applied at GS31 on the percentage area of the upper fully expanded (Leaf 1) leaf affected by powdery mildew in Experiment WKCL2 on 18.5.92.

	Powdery mildew		
treatment	Apollo	Riband	Mean
Solid	13.1	8.7	10.9
Liquid	6.4	4.6	5.5
SPF	5.4	3.7	4.5
SP	8.9	7.3	8.1
Solid+F	8.0	5.0	6.5
Solid+Water	10.9	7.0	9.0
Mean	8.8	6.0	

M.S.D.	
applications	2.5
treatments	3.9
S.E.M.	
application	0.55
variety	0.66
variety * application	0.97
applications within var.	0.78
ANOVA	
variety	*
application	***
variety * application	NS
d.f.	20
C.V.%	
variety	15.4
treatments	18.2

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.18. The potassium content in parts per million of the upper fully expanded (Leaf 1) leaf of winter wheat immediately before treatments were applied at GS 31 in Experiment WKCL2.

	Dry Weight			Fresh Weight			Tissue Water Basis		
treatment	Apollo	Riband	Mean	Apollo	Riband	Mean	Apollo	Riband	Mean
Solid	26.3	26.8	26.6	4.9	5.0	5.0	6.1	6.2	6.1
Liquid	28.9	27.4	28.2	5.5	4.5	5.0	6.8	5.4	6.1
SPF	24.9	29.4	27.1	4.5	5.4	5.0	5.5	6.7	6.1
SP	25.7	24.4	25.1	4.5	4.4	4.5	5.5	5.4	5.4
Solid+F	26.3	26.0	26.2	5.2	4.7	4.9	6.4	5.7	6.1
Solid+Water	27.5	26.4	26.9	4.3	5.4	5.4	6.6	6.9	6.7
Grand Mean		26.7			5.0			6.1	
M.S.D.		5.13			2.01			1.48	
S.E.M.									
variety		0.84			0.27			0.13	
application		1.03			0.38			0.38	
variety * application		1.45			0.51			0.54	
ANOVA									
variety		NS			NS			NS	
application		NS			NS			NS	
variety*application		NS			NS			NS	
D.F.		20			20			20	
C.V.%		9.4			13.4			15.4	

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.19. The potassium content in parts per million of the upper fully expanded (Leaf 1) leaf of winter wheat one day after treatments were applied at GS 31 in Experiment WKCL2.

	Dry Weight			Fresh Weight			Tissue Water Basis		
treatment	Apollo	Riband	Mean	Apollo	Riband	Mean	Apollo	Riband	Mean
Solid	26.7	30.2	28.4	4.9	5.1	5.0	6.1	6.1	6.1
Liquid	30.7	30.0	30.3	5.6	5.2	5.4	6.9	6.3	6.6
SPF	36.5	31.3	33.9	6.1	6.1	6.0	7.4	7.1	7.2
SP	28.5	35.5	32.0	5.1	5.1	5.7	6.2	7.5	6.9
Solid+F	30.0	30.8	30.4	5.3	5.1	5.2	6.4	6.1	6.3
Solid+Water	33.0	33.0	33.0	5.8	5.7	5.8	7.0	6.9	7.0
Grand Mean		31.4			5.0			6.7	
M.S.D.		9.9			1.51			1.26	
S.E.M.									
variety		1.74			0.31			0.39	
application		2.13			0.38			0.67	
variety * application		4.08			0.54			0.91	
ANOVA									
variety		NS			NS			NS	
application		NS			NS			NS	
variety * application		NS			NS			NS	
D.F.		20			20			20	
C.V.%		16.6			13.4			15.4	

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.20. The potassium content in parts per million of the upper fully expanded (Leaf 1) leaf of winter wheat four days after treatments were applied at GS 31 in Experiment WKCL2.

	Dry Weight			Fresh Weight			Tissue Water Basis		
Treatment	Apollo	Riband	Mean	Apollo	Riband	Mean	Apollo	Riband	Mean
Solid	31.8	24.7	28.3	4.09	3.5	3.8	4.7	4.0	4.4
Liquid	27.7	27.7	30.8	4.23	5.4	4.8	5.0	6.4	5.7
SPF	25.0	23.7	24.3	3.57	3.3	3.4	4.2	3.8	4.0
SP	22.3	22.3	25.5	3.14	4.4	3.8	3.7	5.2	4.5
Solid+F	22.3	23.7	23.0	3.16	3.3	3.3	3.7	3.9	3.8
Solid+Water	25.0	24.0	24.5	3.12	3.5	3.3	3.6	4.1	3.8
Grand Mean		26.06			3.7			4.4	
M.S.D.		11.36			2.03			1.71	
S.E.M.									
variety		1.71			0.25			0.30	
application		2.01			0.30			0.37	
variety * application		2.96			0.43			0.52	
ANOVA									
variety		NS			NS			NS	
application		NS			**			**	
variety*application		NS			NS			NS	
D.F.		20			20			20	
C.V.%		19.7			19.9			20.6	

Solid = an application of 33.3 kg/ha solid potassium chloride
Liquid = an application of 33.3 kg/ha potassium chloride as a foliar spray in 220 l/ha water.
SPF = 100 kg/ha potassium chloride powder applied at GS31 + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™
SP = 100 kg/ha potassium chloride powder applied at GS32
Solid + Water = an application of 33.3 kg/ha solid potassium chloride + a foliar spray of 220 l/ha water.
Solid +F = an application of 33.3 kg/ha solid potassium chloride + 625ml/ha Sanction ™ + 750 ml/ha BAS 464™

Table 4.21. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the flag leaf (Leaf 1) of winter wheat affected by powdery mildew on 4.8.92.

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	1.6	1.3	1.5
Liquid	Solid	Solid	1.8	2.8	3.3
Solid	Liquid	Solid	2.3	2.5	2.4
Solid	Solid	Liquid	1.8	1.5	1.7
Liquid	Liquid	Solid	2.0	1.5	1.8
Solid	Liquid	Liquid	2.9	1.3	2.1
Liquid	Solid	Liquid	1.3	0.9	1.1
Liquid	Liquid	Liquid	1.1	1.7	1.4
SPF			5.8	7.7	6.7
SP			5.7	6.8	6.3
Solid +F	Solid +F	Solid +F	6.1	7.2	6.7
Solid + Water			5.4	6.7	6.0

S.E.M for factor means 0.22
S.E.M. for two way factorial interaction 0.31
S.E.M. for three way factorial interaction 0.44
S.E.M. for four way factorial interaction 0.63

M.S.D. 7.49

ANOVA	
variety	*
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF v SP	NS
d.f.	46
C.V.%	6.6

For treatment details see table 4.10

Table 4.22. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the penultimate leaf (leaf 2) of winter wheat affected by powdery mildew on 4.8.92.

Variety	Apollo	Riband	Mean
Treatment			
GS 31 GS 39 GS 59			
Solid Solid Solid	9.0	5.4	7.2
Liquid Solid Solid	12.6	14.3	13.4
Solid Liquid Solid	8.8	4.8	6.8
Solid Solid Liquid	12.1	12.5	12.3
Liquid Liquid Solid	8.1	8.5	8.3
Solid Liquid Liquid	9.5	12.9	11.2
Liquid Solid Liquid	12.1	10.7	11.4
Liquid Liquid Liquid	19.8	8.5	14.2
SPF	25.1	15.1	20.1
SP	15.1	13.6	14.4
Split Solid +F	15.4	3.2	9.3
Split Solid + Water	10.3	20.5	15.4

S.E.M for factor means 1.81
S.E.M. for two way factorial interaction 2.55
S.E.M. for three way factorial interaction 3.61
S.E.M. for four way factorial interaction 5.11

M.S.D. 32.48

ANOVA	
variety	NS
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SP vSP	NS
d.f.	46
C.V.%	48.5

For treatment details see table 4.10

Table 4.23. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the flag leaf (leaf 1)) of winter wheat affected by *Septoria tritici* on 4.8.92.

Variety	Apollo	Riband	Mean
Treatment			
GS 31 GS 39 GS 59			
Solid Solid Solid	3.0	6.6	4.8
Liquid Solid Solid	4.2	9.3	6.8
Solid Liquid Solid	6.3	7.2	6.8
Solid Solid Liquid	5.9	7.9	6.9
Liquid Liquid Solid	6.4	7.9	7.2
Solid Liquid Liquid	5.4	7.8	6.6
Liquid Solid Liquid	4.7	8.3	6.5
Liquid Liquid Liquid	3.3	6.5	4.9
SPF	4.0	4.5	4.3
SP	3.7	7.3	5.5
Split solid +F	3.2	1.9	2.6
Split solid + Water	3.9	7.5	5.7

S.E.M for factor means	0.56
S.E.M. for two way factorial interaction	0.80
S.E.M. for three way factorial interaction	1.13
S.E.M. for four way factorial interaction	1.59

M.S.D. 7.49

ANOVA	
variety	NS
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SP vSP	NS
d.f.	46
C.V.%	49

For treatment details see table 4.10

Table 4.24. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the penultimate leaf (leaf 2) of winter wheat affected by *Septoria tritici* on 4.8.92.

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	5.8	4.2	5.0
Liquid	Solid	Solid	3.1	4.8	4.0
Solid	Liquid	Solid	3.8	5.8	4.8
Solid	Solid	Liquid	3.8	5.1	4.5
Liquid	Liquid	Solid	4.6	6.3	5.5
Solid	Liquid	Liquid	7.0	6.2	6.6
Liquid	Solid	Liquid	3.1	17.7	10.4
Liquid	Liquid	Liquid	4.6	8.5	6.6
SPF			9.9	10.7	10.3
SP			15.2	9.9	12.6
Split solid +F			7.3	7.2	7.3
Split solid + Water			7.7	7.1	7.4

S.E.M for factor means	1.04
S.E.M. for two way factorial interaction	1.48
S.E.M. for three way factorial interaction	2.09
S.E.M. for four way factorial interaction	2.95

M.S.D. 8.45

ANOVA	
variety	***
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	*
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF vSP	**
d.f.	46
C.V.%	74

For treatment details see table 4.10

Table 4.25. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the flag leaf (leaf 1) of winter wheat affected by brown rust *Puccinia recondita* on 4.8.92.

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	1.1	0.4	0.8
Liquid	Solid	Solid	0.8	0.6	0.7
Solid	Liquid	Solid	1.5	0.5	1.0
Solid	Solid	Liquid	1.8	0.6	1.2
Liquid	Liquid	Solid	2.8	0.7	1.8
Solid	Liquid	Liquid	1.6	1.1	1.4
Liquid	Solid	Liquid	0.9	1.0	1.0
Liquid	Liquid	Liquid	0.7	0.8	0.8
SPF			0.6	0.2	0.4
SP			2.3	0.7	1.5
Split solid +F			0.4	0.5	0.4
Split solid + Water			2.2	0.4	0.3

S.E.M for factor means	0.17
S.E.M. for two way factorial interaction	0.24
S.E.M. for three way factorial interaction	0.34
S.E.M. for four way factorial interaction	0.48

M.S.D. 4.37

ANOVA	
variety	*
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF vSP	NS
d.f.	46
C.V.%	83

For treatment details see table 4.10

Table 4.26. The effect of the form and timing of potassium chloride fertiliser application in Experiment WKCL2 on the percentage area of the penultimate leaf (leaf 2) of winter wheat affected by brown rust *Puccinia recondita* on 4.8.92.

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	1.5	0.5	1.0
Liquid	Solid	Solid	1.1	4.9	3.0
Solid	Liquid	Solid	2.5	0.5	1.5
Solid	Solid	Liquid	0.6	0.7	0.7
Liquid	Liquid	Solid	1.7	0.8	1.3
Solid	Liquid	Liquid	1.6	0.6	1.1
Liquid	Solid	Liquid	0.8	0.9	0.9
Liquid	Liquid	Liquid	0.4	1.4	0.9
SPF			1.5	0.7	1.1
SP			0.6	0.8	0.7
Split solid +F			1.3	0.9	1.0
Split solid + Water			1.1	1.9	1.5

S.E.M for factor means	0.36
S.E.M. for two way factorial interaction	0.51
S.E.M. for three way factorial interaction	0.72
S.E.M. for four way factorial interaction	1.01
M.S.D. 7.49	

ANOVA	
variety	NS
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF vSP	NS
d.f.	46
C.V.%	49

For treatment details see table 4.10

Table 4.27. The effect of the form and timing of potassium chloride fertiliser application on the yield of winter wheat adjusted to 15% moisture content in Experiment WKCL2 .

Variety	Apollo	Riband	Mean
Treatment			
GS 31 GS 39 GS 59			
Solid Solid Solid	5.79	6.75	5.40
Liquid Solid Solid	5.55	6.9	6.23
Solid Liquid Solid	5.47	6.65	6.06
Solid Solid Liquid	5.62	6.65	6.12
Liquid Liquid Solid	5.76	6.58	6.17
Solid Liquid Liquid	5.37	6.24	5.81
Liquid Solid Liquid	5.26	6.48	5.87
Liquid Liquid Liquid	5.40	6.78	6.09
SPF	5.78	7.69	6.73
SP	5.69	6.83	6.26
Split solid +F	6.09	7.23	6.66
Split solid + Water	5.36	6.66	6.00

S.E.M for factor means	0.08
S.E.M. for two way factorial interaction	0.12
S.E.M. for three way factorial interaction	0.17
S.E.M. for four way factorial interaction	0.24

M.S.D. 1.5

ANOVA	
variety	***
GS31	NS
GS39	*
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	*
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	*
Split v SP	NS
SPF vSP	NS
d.f.	46
C.V.%	6.6

For treatment details see table 4.10

Table 4.28. The effect of the form and timing of potassium chloride fertiliser application on the specific weight (kg/hl) of winter wheat in Experiment WKCL2 .

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	75.10	71.88	73.49
Liquid	Solid	Solid	74.30	72.32	73.31
Solid	Liquid	Solid	74.85	72.20	73.53
Solid	Solid	Liquid	74.98	70.78	72.88
Liquid	Liquid	Solid	75.30	71.50	73.40
Solid	Liquid	Liquid	74.72	70.97	72.85
Liquid	Solid	Liquid	75.38	70.88	73.13
Liquid	Liquid	Liquid	75.30	70.80	73.05
SPF			75.45	72.03	73.74
SP			74.83	72.03	73.43
Split solid +F			75.48	71.79	73.64
Split solid + Water			74.20	70.95	72.58

S.E.M for factor means	0.23
S.E.M. for two way factorial interaction	0.32
S.E.M. for three way factorial interaction	0.45
S.E.M. for four way factorial interaction	0.64

M.S.D. 7.72

ANOVA	
variety	NS
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	*
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF vSP	NS
d.f.	46
C.V.%	1.5

For treatment details see table 4.10

Table 4.29. The effect of the form and timing of potassium chloride fertiliser application on the thousand grain weight (g) of winter wheat in Experiment WKCL2.

Variety			Apollo	Riband	Mean
Treatment					
GS 31	GS 39	GS 59			
Solid	Solid	Solid	49.63	47.14	48.39
Liquid	Solid	Solid	47.04	47.36	47.20
Solid	Liquid	Solid	45.94	48.51	47.23
Solid	Solid	Liquid	45.45	48.98	47.22
Liquid	Liquid	Solid	49.58	47.25	48.42
Solid	Liquid	Liquid	46.22	45.88	46.05
Liquid	Solid	Liquid	47.56	48.06	47.81
Liquid	Liquid	Liquid	48.75	46.94	47.85
SPF			50.53	51.47	50.91
SP			47.06	46.51	46.79
Split solid +F			49.48	49.54	49.51
Split solid + Water			48.93	48.24	48.56

S.E.M for factor means	0.62
S.E.M. for two way factorial interaction	0.88
S.E.M. for three way factorial interaction	1.25
S.E.M. for four way factorial interaction	1.77

M.S.D. 7.72

ANOVA	
variety	NS
GS31	NS
GS39	NS
GS59	NS
GS31.GS39	NS
GS31.GS59	NS
GS39.GS59	NS
GS31.GS39.GS59	NS
var*GS31	NS
var*GS39	NS
var*GS59	NS
var*GS31.GS39	NS
var*GS31.GS59	NS
var*GS39.GS59	NS
var*GS31.GS39.GS59	NS
Split v SP	NS
SPF vSP	*
d.f.	46
C.V.%	1.5

For treatment details see table 4.10

4.2.5. Discussion

The data gathered in the two weeks following the growth stage 31 applications suggested that the application of potassium chloride to wheat as a foliar spray reduced the percentage leaf area affected by powdery mildew compared with equivalent dressings of solid fertiliser to the soil. The quantity of potassium chloride applied at growth stage 31 appeared to have little or no effect on the severity of the disease despite the soil having low reserves of potassium and this element being widely regarded as an essential element for plant disease resistance. This suggested that the plants were adequately supplied with potassium for maximum exhibition of its disease resistance at the lower rate of fertiliser. However the foliar spray of potassium chloride supplying 33.3 kg/ha of potassium chloride fertiliser resulted in greater control than that provided by soil dressings which supplied equivalent or higher rates of the compound. This suggested that the properties of the foliar spray differed from those of the soil applications.

The tissue analysis indicated that uptake of potassium probably did occur and that this uptake was faster with foliar application than soil applied treatments. Over the short term (one to three weeks) the rate of uptake by the plant roots may have provided a limitation to the intake of potassium by the plant at all of the rates at which it was applied to the soil. A foliar spray may have resulted in a greater uptake of potassium than a soil application by-passing the roots and may therefore have made more potassium available at the cellular level than a soil application possibly could. By-passing the selective uptake mechanisms of the plant roots, the foliar applications may have resulted in a greater concentration of potassium in the leaf cells. This was supported by the tissue analysis carried out.

It appeared possible that the elevated potassium levels, on a fresh weight and tissue water basis, caused by the foliar application of potassium chloride

solution were related to reductions in the leaf area affected by *E. graminis*. However, this link was not proved directly and further investigation under controlled conditions was thought necessary before confident conclusions could be made. If the increased uptake of ions did occur leading to higher concentrations of potassium in the cell water then the possibility existed for a short term increase in the osmotic potential of the cell sap. Such an increase may have proved deleterious to the establishment of a biotrophic relationship between parasite and host. In addition a strong salt solution on the leaf surface may have resulted in the lysis of mildew spores or germ tubes.

The application of water had no significant effect on the severity of the disease. The absence of any effect on powdery mildew was surprising in view of the published work relating to the loss of viability of *E. graminis* spore when exposed to free water (Corner, 1935; Zaracovitis, 1966). However the small volume of water applied in this experiment was probably little different to, or less than, the dew intercepted by a cereal canopy each night. It is not unreasonable to suggest that *E. graminis* has probably evolved to tolerate exposure to such small quantities of free water for limited periods of time. However the application of water with the split dressings of potassium chloride did conclusively prove that the disease retarding properties of foliar applied potassium chloride is due to the properties of the solution and not the water component alone.

The absence of a varietal interaction refuted Huber's (1980) suggestion that varieties with different resistance levels differ in their response to potassium nutrition. This suggests that the control of disease provided by the foliar potassium chloride is due to either the foliar fertiliser having a direct effect on the pathogen or providing the components for the plant to produce broad resistance chemicals rather than eliciting cultivar specific genetically determined defence responses from the host. This experiment therefore did not provide support for

The absence of a varietal interaction refuted Huber's (1980) suggestion that varieties with different resistance levels differ in their response to potassium nutrition. This suggests that the control of disease provided by the foliar potassium chloride is due to either the foliar fertiliser having a direct effect on the pathogen or providing the components for the plant to produce broad resistance chemicals rather than eliciting cultivar specific genetically determined defence responses from the host. This experiment therefore did not provide support for Huber's (1980) suggestion that varieties with moderate disease resistance respond better to improved fertiliser practice than those with low disease resistance.

The absence of late season disease control was puzzling when the control had been so good early in the season. However the dry conditions after flag leaf emergence may have contributed to an overall low severity of foliar disease in the crop. At very low disease severity it is difficult to detect differences between treatments and the absence of control by the conventional fungicides backs this view.

The lack of a yield response to any of the treatments which reduced disease was thought to be attributable to two sources. Firstly the effects on yield of the different disease severity after the growth stage 31 treatments may have been negated by compensatory growth later in the season. Secondly the low levels of disease on the flag leaf meant that any differences between treatments were insignificant so no response to disease control could have been expected. The control of disease on the flag leaf was expected to be the most likely treatment to give a yield response because this leaf contributes fifty percent of the total synthate used in the production of grain (Anon, 1981).

The absence of scorch in this experiment, which contrasted with the findings of Kettlewell *et al.* (1990), was attributed to the use of low spray volumes which minimised the residence time of liquid on the leaf surface. The water in the solutions quickly evaporated leaving a salt deposit on the leaf surface.

This was probably re-dissolved overnight in the dew and then taken up. This slow uptake was reflected by the absence of significant differences in tissue water potassium ion concentration between the treatments one day after application. It was believed that this de-watering and re-wetting of the salt led to slower uptake and reduced osmotic flux in the leaf. This resulted in lower phyto-toxicity.

4.3.0 Combined Discussion

It would appear that potassium chloride applied to the foliage as a liquid spray will reduce the leaf area of wheat affected by *E. graminis* and *S. tritici* compared to an equivalent application of solid fertiliser to the soil.

These effects consistently took between seven and fourteen days to become apparent. This was believed to indicate that the foliar potassium chloride reduced leaf area affected by killing spores and young hyphae on the leaf surface or by disrupting the infection process.

It was thought that the control was achieved by an activity of the ions in the solution against the pathogens on the leaf surface or by entering the leaf and altering the internal environment of the leaf.

It was highly likely that the application of a solution with a high osmotic potential to the leaf would have caused an efflux of water from spores or hyphae on the leaf surface, leading to their eventual collapse. It has been shown that the uptake of potassium (Chamel, 1988) by leaves is high. Tissue analysis in the second field experiment indicated that foliar applications of potassium chloride resulted in elevated concentrations of potassium ions in the leaf water. This may have altered the osmotic potential of the cell sap rendering the epidermal cells of the leaf less suitable for the pathogen.

The absence of leaf scorch in the experiments was attributed to the low volumes of spray applied compared to other experiments (Kettlewell *et al.* 1990). It was thought that the rapid disappearance of the liquid from the leaf surface was

by evaporation leaving the salts on the leaf surface to be slowly re-dissolved in dew, leading to a very slow uptake which maintained the cellular concentration of ions below phyto-toxic levels.

The absence of a varietal interaction suggested that the reduction in leaf area affected was achieved by a physico-chemical effect, direct toxicity to the pathogen or enhanced production of broad chemical resistance chemicals rather than the elicitation of genetically determined narrow genic resistance mechanisms.

The effect of the quantity of fertiliser applied around growth stage 32 was unclear. In one year increased fertiliser reduced disease but in the second experiment on a less fertile site the rate had no effect. Therefore it was concluded that the rate of fertiliser applied at this timing was of marginal importance.

The absence of consistent late season disease control by foliar fertiliser was surprising in light of the early season control. Control of disease was never recorded from an application after full ear emergence. In view of the lag in time before differences in disease severity manifested themselves between treatments earlier in the season it may be that this application time is too late to reveal any effects. Differences between treatments applied around growth stage 39 occurred in one year only. In the second experiment the low levels of disease present reduced the likelihood of obtaining differences.

The absence of any effect on yield by the foliar potassium chloride applications when disease control was evident is surprising. Fungicide treatment increased yields in the first experiment but not in the second. This suggested that late season disease was not a significant factor affecting yield in the second experiment.

The absence of yield responses to the control of disease at early stem extension was surprising in view of the work by Gaunt, Lim and Thomson (1982) who indicated that *Septoria tritici* could affect the yield of wheat throughout the

growing season. Early season infection by *Septoria tritici* was shown to decrease yield by affecting yield components developing after the incidence of the disease (Thomson and Gaunt, 1986) and was more significant than late season epidemics. Evidence of this situation with regard to *E. graminis* on wheat was unavailable.

However Thomson and Gaunt (1986) did observe some compensation for reductions in yield potential caused by infections occurring early in the growing season which was not surprising because the ear, flag, second and third leaves contribute over eighty percent of the total grain yield of a cereal crop (Anon, 1981). Therefore the direct effect of disease control at stem extension would be small and insignificant compared to other effects. The significant reduction of disease severity by the flag leaf spray in the first experiment did not lead to increased yield. This was unusual in view of the high contribution this leaf makes to yield. It was suggested that heavy rain leading to water logging of the soil around anthesis may have prevented a yield response.

Chapter 5 - The Effect of Potassium Chloride Solution Concentration on *Erysiphe graminis*, The Cause of Cereal Powdery Mildew, *in vivo*, on Wheat

5.0 Introduction

Potassium chloride solutions applied to the foliage of cereal plants have been observed to reduce the severity of cereal diseases in the field on several occasions. It has been reported that the leaf area affected by *Erysiphe graminis* DC. f. sp. *tritici*, the pathogen causing the disease powdery mildew of wheat, was reduced by applications of potassium chloride both early in the season and late in the season (Cook, Kettlewell and Parry, 1993; Kettlewell *et al.*,1990). Kettlewell *et al.* (1990) used concentrations ranging from 5.8% to 23% w/v. The field experiments conducted for this project used a single concentration of 15.9% w/v potassium chloride (Cook *et al.*, 1993).

The scarcity of information on the response of the pathogen to different concentrations of potassium chloride solution applied as a foliar spray was considered to be a major problem in understanding the mechanism of how the fertiliser reduced disease. This experiment had to be conducted *in vivo* as *E. graminis* cannot be cultured on artificial media. This precluded the more usual initial investigations of fungicide activity by examining colony growth on amended media.

5.1 Objective

To determine the effect of different concentrations of foliar applied potassium chloride on the leaf area of wheat affected by *E. graminis*.

5.2 Materials and Methods

The glasshouse experiment was repeated three times with minor variations in design and plant material used. Plants were grown as described in general materials and methods. The plants were removed from the propagator at the commencement of the experiment and selected for uniformity. The plants were at the four fully expanded leaf stage (Experiments GH1 and GH2) or three leaf stage (Experiment GH3). After selection plants were randomly allocated to treatments.

Stock pots were inoculated three weeks before the start of the experiments, following preparation as described in general materials and methods, and maintained at a minimum temperature of 15 ^{o}C by day and 5 ^{o}C by night on gravel beds which were regularly wetted to maintain humidity. Each variety was inoculated with spores from pustules of the same variety. Each experiment was of a randomised block design with ten treatments, an untreated control, a control sprayed with distilled water and eight concentrations (2,4,6,8,10,12,14 and 16% w/v) of foliar applied potassium chloride fertiliser (Analar grade; BDH, Poole, England). In experiment GH1 there were two blocks each containing ten replicates. In experiments GH2 and GH3 there were ten blocks each containing one replicate. One replicate consisted of one pot containing one wheat plant. The plants, except the untreated control, were sprayed using a precision pot sprayer, as described in general materials and methods, fitted with a medium flat fan jet (015-F80, Lurmark, Cambridge, England) at approximately 3 bar and a rate equivalent to 200l/ha. The application rate was calibrated by measuring the flow rate of the spray head and using tables to convert the value to volume per unit area.

After treatment the plants were set out in randomised blocks on a glasshouse bench over dampened gravel. After one hour the leaf surface bore no visible water and the experiment was inoculated by shaking heavily infected stock

pots over the plants. The stock pots had been shaken the previous night to remove any spore chains.

After two weeks the experiments were assessed for percentage area affected by *E. graminis* using visual inspection in conjunction with ADAS disease assessment keys (Anon, 1976) following practice using computer training aids.

In experiments GH1 and GH2 the fourth leaf from the stem base was assessed. In experiment GH3 the third leaf from the stem base was assessed. In Experiment GH1 only the upper surface of the selected leaf was assessed. In Experiments GH2 and GH3 both the upper and lower leaf surfaces of the selected leaves were assessed.

A small stock of plants was retained in the spore free propagation unit and inspected to two weeks after the start of the experiment to ensure that the plants had been free of disease at the start of the experiment. The results were analysed by analysis of variance with polynomial contrasts. (Ridgeman, 1975).

5.3 Results

In all cases the inoculations produced an adequate level of infection for assessment to be possible.

Potassium chloride had a significant effect (P=0.05) on the area of the leaf affected by *E. graminis* (Figure 9). In all three experiments there was a strong quadratic trend (P=0.05) in the data. This applied to both the upper and lower leaf surfaces. The optimum control was achieved by about 10% w/v potassium chloride. Above this concentration the disease severity generally increased.

The disease response curve for Experiment GH3, where the plants were younger, had a much more distinctly quadratic nature than those of Experiments GH1 and GH2.

The plants retained in the spore free propagator did not show any infection after three weeks indicating that the plants were free of infection at the start of the experiment. Phytotoxicity symptoms, manifested as leaf tissue necrosis or scorch were not observed in any of the experiments. The results are summarised in Table 5.1.

Figure 9 Experiments GH1,GH2, and GH3 The effect of increasing concentration of foliar applied potassium chloride solution on the percentage area of unvernalised winter wheat leaves, cv. Apollo (GH1 and GH3) and cv. Riband (GH2) affected by *Erysiphe graminis* in glasshouse experiments. Assessments made two weeks after treatment on the upper side of the fourth leaf (GH1 and GH2) or third leaf (GH3) from the stem base (bars = S.E.M.)

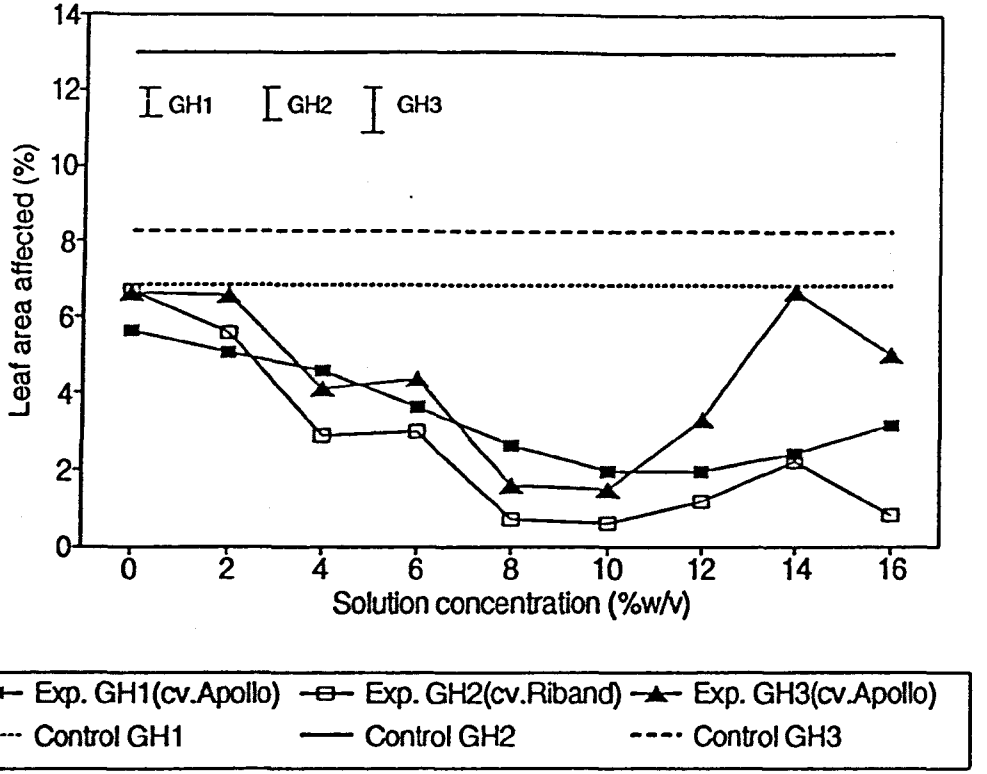


Table 5.1 Experiments GH1,GH2, and GH3. The effect of increasing concentration of foliar applied potassium chloride solution on the percentage area of unvernalised winter wheat leaves, cv.Apollo (GH1 and GH3) and cv. Riband (GH2) affected by *Erysiphe graminis* in glasshouse experiments. Assessments made two weeks after treatment on the fourth leaf (GH1 and GH2) or third leaf (GH3) from the stem base.

Experiment	GH1	GH2		GH3	
Leaf side	adaxial	adaxial	abaxial	adaxial	abaxial
Treatment					
Control	6.9	13.0	7.7	8.3	2.8
0%	5.7	6.7	3.4	6.7	1.8
2%	5.1	5.6	1.3	6.6	1.3
4%	4.6	2.9	1.5	4.1	2.2
6%	3.7	3.0	1.3	4.4	1.7
8%	2.7	0.7	1.6	4.6	3.3
10%	1.1	1.6	1.2	1.5	1.5
12%	2.0	1.2	0.6	3.3	1.4
14%	2.4	2.1	1.5	6.7	0.9
16%	3.5	0.8	0.4	5.0	1.0
ANOVA					
Treatment	***	***	**	*	*
Linear	**	***	***	NS	NS
Quadratic	*	*	*	*	*
Cubic	NS	NS	NS	NS	NS
S.E.M	0.90	1.28	1.13	1.01	0.67
M.S.D	4.18	5.94	5.23	4.70	3.11
d.f.	164	78	78	78	78
C.V	128	143	143	118	118

5.4 Discussion

The foliar application of potassium chloride solution to wheat plants shortly before inoculation reduced the leaf area affected by *E. graminis*. This was in agreement with the field experiments (Cook *et al.*, *1993;* Kettlewell *et al.*, 1990). In all cases the optimal control was achieved by concentrations of approximately 10% w/v. However, the effectiveness of potassium chloride as a disease control agent declined at higher concentrations as reflected by the quadratic nature of the disease response curve.

It was suspected that the initial effect of potassium chloride solutions would be to plasmolyse *E. graminis* spores on the leaf surface. However the coverage of the leaf by spray droplets was not perfect resulting in areas of the leaf not being treated. The surviving spores would then infect the leaf. However the uptake of potassium chloride was expected to change the osmotic potential of the cytoplasm of the plant cells. Lower concentrations of potassium chloride may have altered cell osmotic potential, and possibly cell metabolism, enough to prevent the establishment of infection. However the influx of large quantities of potassium and chloride ions supplied by the more concentrated solutions may have caused severe, but non lethal, cellular dysfunction. This could have lowered the production of defensive compounds in the leaf as a whole, making those areas not receiving droplets more susceptible to infection. The plants in experiment GH3 were younger and less developed than those in the other experiments. It is possible that these plants were less able to tolerate the application of high concentrations of potassium chloride solution. This could have resulted in greatly enhanced susceptibility giving the more pronounced quadratic trend seen in this experiment.

Chapter 6 - The Effects of Potassium Chloride on the leaf area of winter wheat cv. Apollo affected by Erysiphe graminis when Applied to the Soil or as a Foliar Spray at Four Times relative to Inoculation (Experiments GH4a and GH4b).

6.0 Introduction

The field experiments, detailed in Chapter 4, indicated that the use of foliar-applied in place of solid, soil-applied potassium chloride fertilisers resulted in lower leaf areas affected by *E. graminis.*

It was considered important to confirm these findings in the glasshouse. In the development of a system which utilised potassium chloride to control foliar cereal disease it was considered essential to know whether the timing of fertiliser application relative to inoculation had any effect on its efficacy. It was decided that a single experiment with a factorial design could provide information on both of these points.

6.1 Objectives

1) To compare the effect of foliar and soil applied potassium chloride solutions on the leaf area of wheat plants affected by *E. graminis* in the glasshouse.
2) To determine the effect of fertiliser application timing relative to inoculation on the leaf area of wheat plants affected by *E. graminis.*

6.2 Materials and Methods

Wheat plants (cv. Apollo) were produced according to the protocol described in the general materials and methods. The seeds were chitted for four days at 20°C before planting and the plants were twenty eight days old at the commencement of the experiment. The plants had three fully expanded leaves at the commencement of

the experiment and were selected for uniformity and healthy appearance. Plants were allocated to treatments by random numbers.

Experiment GH4a was of a factorial design with two factors which were applications and timing of applications. There were three levels of applications, a soil drench of potassium chloride, a foliar spray of potassium chloride supplying an equivalent quantity of potassium chloride per plant or a foliar spray of distilled water. Four levels of timing were used, three and seven days pre and post inoculation. Ten replicates were used. The experiment was blocked with one replicate of each treatment per block. The blocks were laid out in a two by five grid pattern. Within each block the pots were set out in a three by four grid pattern. Position was allocated by the use of random numbers.

The foliar sprays were applied using a precision pot sprayer fitted with a medium flat fan jet (015-F80, Lurmark, Cambridge, England) at approximately 3 bar and a rate equivalent to 200 l/ha as described in general materials and methods. The foliar potassium chloride spray was of a 10% w/v solution. The soil application consisted of 1.34 ml per pot of 0.1% w/v potassium chloride solution which supplied an equivalent amount of potassium chloride as the foliar spray. All solutions were made by dissolving the salt in distilled water (Analar grade; BDH, Poole, England).

After treatment the plants were set out on a glasshouse bench over damped gravel in randomised blocks. The experiment was inoculated by shaking heavily infected stock pots (cv. Apollo), produced as detailed in general materials and methods, over the plants. The stock pots had been shaken the previous night to remove any spore chains.

The experiment was assessed for percentage area of the third leaf from the base of the plant affected by *E. graminis* fourteen days after inoculation using visual inspection in conjunction with ADAS disease assessment keys (Anon 1976) following

practice using computer training aids (Distrain™). A small stock of plants retained in the spore free propagation unit was inspected to ensure that the plants had been free of disease at the start of the experiment. The results were analysed by analysis of variance with polynomial contrasts (Ridgeman, 1975).

A second experiment (GH4b) was conducted to further support the conclusions resulting from the first. The second experiment was identical to the first except that the soil treatments were omitted. The plants had been grown for forty two days at ambient air temperature and had four fully expanded leaves at inoculation. The experiment was laid out as ten blocks in a two by five grid pattern. Within each block the pots were laid out in a two by four grid pattern.

6.3. Results.

Experiment GH4a.

With respect to both the upper and lower sides of the third leaf the type of application had significant effects on the percentage leaf area affected by *E. graminis* (P=0.01). At each time of application foliar potassium chloride solutions reduced the leaf area affected by the pathogen compared with soil applications or the water spray (Figures 10 and 11). These reductions were often in excess of fifty percent. There was no clear pattern in the relative effects of the water spray and soil applied potassium chloride treatments. Full results are shown in Appendix B2.

The percentage area of the lower side of the leaf affected by *E. graminis* declined in a linear manner as the treatments were applied later relative to the time of inoculation(P=0.05). There was no significant interaction between timing and

application except for a cubic response on the lower side of the leaf (P=0.05) apparently due to variation in the response to timing of the water treatment (Appendix B3).

Experiment GH4b.

In Experiment GH4b only data appertaining to the upper side of the third leaf from the stem base was analysed because this was the only data set exhibiting a leaf area affected high enough for assessment. In this experiment, timing had no significant effect upon the effectiveness of the potassium chloride fertiliser at reducing disease. The results showed that potassium chloride reduced the percentage leaf area affected by powdery mildew from 5.6% to 1.2% (P=0.001) (Figure12). Full results are shown in Appendix B4.

Figure 10. Experiment GH4a. The percentage area of the upper side of the third leaf from the stem base of winter wheat, cv. Apollo, affected by *Erysiphe graminis* following treatment with a 10% w/v potassium chloride solution applied to the plants as a foliar spray or a soil drench. A foliar spray of water was applied as a control. The treatments were applied either 7 or 3 days before or after inoculation. Disease was assessed 14 days after inoculation. (bar=S.E.M.)

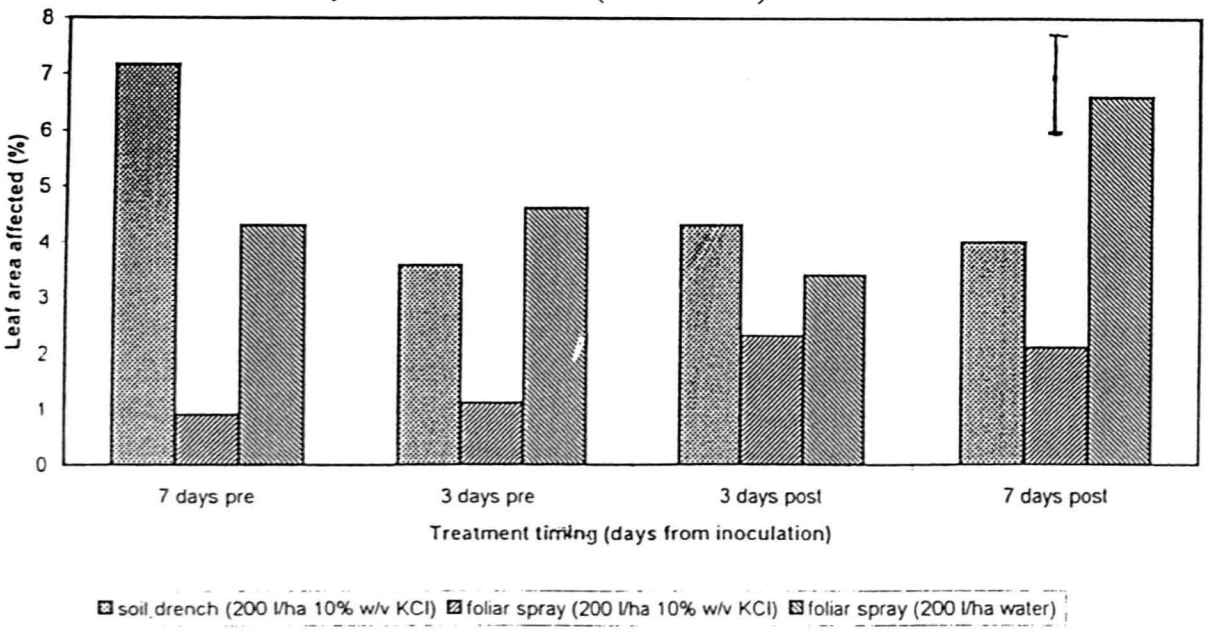


Figure 11. Experiment GH4a. The percentage area of the lower side of the third leaf from the stem base of winter wheat, cv. Apollo, affected by *Erysiphe graminis* following treatment with a 10% w/v potassium chloride solution applied to the plants as a foliar spray or a soil drench. A foliar spray of water was applied as a control. The treatments were applied either 7 or 3 days before or after inoculation. Disease was assessed 14 days after inoculation. (bar=S.E.M.)

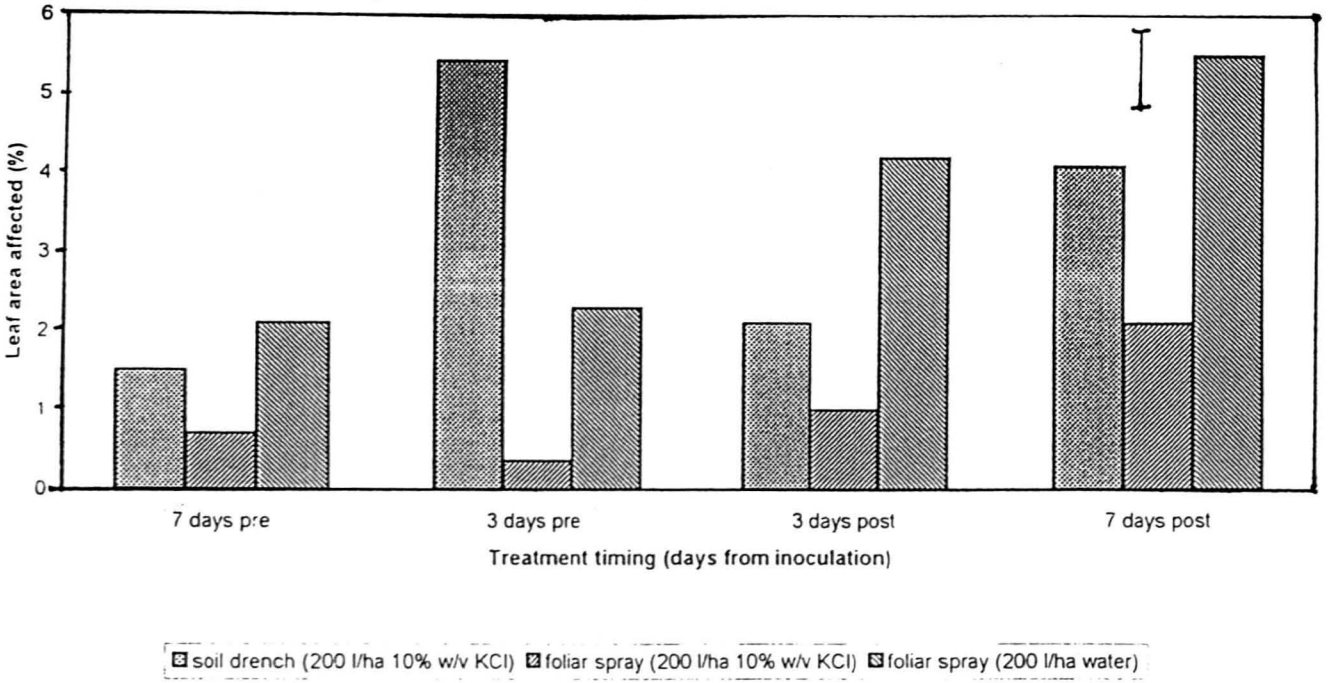
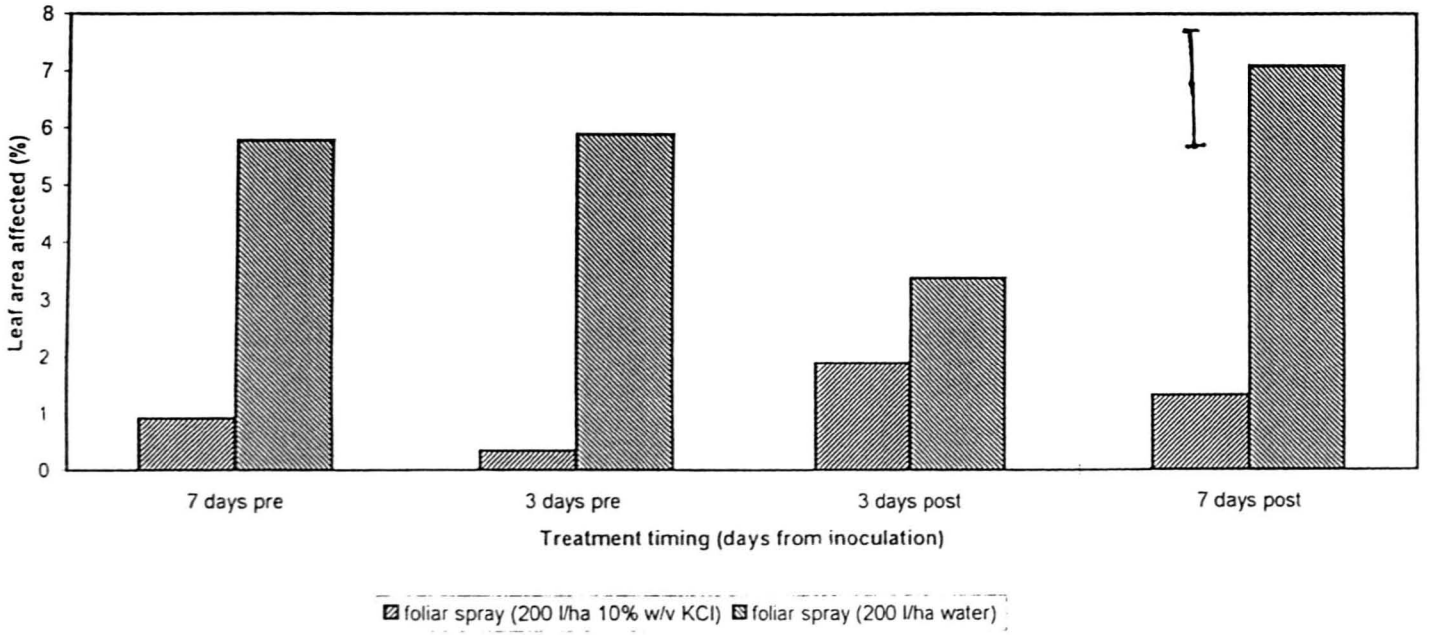


Figure 12. Experiment GH4b. The percentage area of the upper side of the third leaf from the stem base of winter wheat, cv. Apollo, affected by *Erysiphe graminis* following treatment with a 10% w/v potassium chloride solution applied to the plants as a foliar spray . A foliar spray of water was applied as a control. The treatments were applied either 7 or 3 days before or after inoculation. Disease was assessed 14 days after inoculation. (bar=S.E.M.)



6.5. Discussion

Potassium chloride, applied as a foliar spray, was the only treatment in the experiments which consistently reduced the leaf area of winter wheat affected by *Erysiphe graminis*.

The lack of effect of the foliar spray of water confirmed that it was some property of the potassium chloride solution which was responsible for the control of the powdery mildew.

The application of an equivalent quantity of potassium chloride as a soil drench did not have the same effect as a foliar fertiliser spray. This indicated that potassium chloride can only influence powdery mildew development when applied directly to the leaves. It was possible that either potassium or chloride ions were not taken up and translocated to the leaves quickly enough to influence the percentage leaf area affected by powdery mildew. The effect of potassium chloride on the leaf diseases of cereals might be dependent upon the direct placement of the ions on the leaf surface whether or not they were subsequently taken up. The alternative was that some property of the solution itself, unrelated to the ions in that solution, inhibited the development of the powdery mildew. This inhibition might have been due to direct effects on the pathogen or indirectly by altering the physiological state of the leaf.

The timing of application, within 7 days of inoculation, did not appear to have a consistent effect on the activity of foliar-applied potassium chloride solution against powdery mildew. This indicated that the solution was both curative and protectant and might have more than one mode of action.

It was decided that the most logical progression of the work would be to investigate the direct effect of potassium chloride solution on the pathogen and variations in the effect when the solution concentrations were changed and to identify

whether or not potassium chloride was an essential component of the solution. This could then be followed by a more detailed study of the mode of action.

Chapter 7 - The Effect of Potassium Chloride Solution Concentration on *Erysiphe graminis, in vitro* (Experiment SO1).

7.0 Introduction

The hyphae of cucumber powdery mildew (*Sphaerotheca fuliginea*) have been shown to be severely damaged by the application of foliar sprays of potassium chloride and other salt solutions. It was suggested that the application of solutions of high osmotic potential may have caused a net outflow of water from the hyphae resulting in their collapse (Weeds, Long, Ganeshanaddam, Hopcroft and Bennett, 1993). It can be inferred from these observations that the activity of potassium chloride against *E. graminis* may be, at least partly, due to an osmotic effect on the fungal cells.

Studies of *E. graminis in vitro* have the advantage that the removal of the host greatly simplifies the system and enables the study of one particular variable on the development of the pathogen while keeping the rest of the system constant. As this pathogen is an obligate biotroph it is impossible to grow hyphae on artificial media but studies on the effects of potassium chloride on spore morphology and germination *in vitro* were considered possible.

Germination of *E. graminis* conidia on glass has been shown to occur at any humidity, including zero, but germination is poor below ninety eight percent r.h. (Manners and Housain, 1963). Although high humidity is beneficial to germination, free water is widely reported to be deleterious to the viability of powdery mildew conidia. The internal structure of powdery mildew is reported to collapse in water (Corner, 1935; Zaracovitis, 1966), and up to 50% conidial death is reported to occur within three minutes of immersion. However in a few cases spore suspensions of various powdery mildew species in water have been used in experimental inoculations (Reuveni and Rotem, 1973). Due to the apparent divergence of results it was considered that the study of the effects of potassium chloride

solutions on wheat powdery mildew (*E. graminis*) spores *in vitro* was potentially possible.

The concentration of potassium chloride giving maximum control in the glasshouse experiments was much higher than the normal dose rates of conventional fungicides. This suggested that the high osmotic potential of the potassium chloride solutions may have been responsible, at least in part, for the control observed. To simplify the system it was decided to examine the effects of osmotic potential on the morphology, viability and germination potential of *E. graminis* conidia *in vitro*. In order to do this spores were suspended in solutions of potassium chloride with a range of osmotic potentials. Solutions of equal osmotic potential to the potassium chloride were formulated using polyethylene glycol (PEG), a biologically inactive compound (Heydecker and Gibbins, 1978). Spores were suspended in these solutions for comparative purposes to show whether the effects of the potassium chloride were due solely to osmotic potential or to other mechanisms such as direct toxicity of the chloride ion to *E. graminis*.

7.1 Objective

1) To determine the effect of solutions of differing osmotic potential on *E. graminis* spores *in vitro*.
2) To determine the effect of different osmotica on *E. graminis* spores *in vitro*.

7.2 Materials and Methods

The spores used were from pustules grown on the leaves of wheat plants grown singly in FP7 (Plantpak, Maldon, England) pots containing soil-less compost as detailed in general materials and methods. The plants, cv. Apollo, were inoculated three weeks before the experiment commenced and maintained at a minimum temperature of 15 ^{o}C by day and 5 ^{o}C by night on gravel beds which were regularly

wetted to maintain humidity. Spores were taken from plants which had been shaken approximately seven hours before the collection of spores to remove spore chains.

The *in vitro* experiment was a randomised block design with two factors: osmotica at two levels, and osmotic potential of solution at five levels. Treatments were pure deionised distilled water (control) and solutions of 12.83, 25.65, 38.48, 51.31 and 64.14 bar osmotic potential made up using either potassium chloride (Analar, BDH, Poole, Dorset) or polyethylene glycol (PEG) (BDH, Poole, Dorset). These osmotic potentials were equivalent to 2,3,6,8 and 10% w/v potassium chloride and 10.8, 21.5, 32.7, 42.90 and 53.6% w/v PEG solutions. All solutions were made up using sterile distilled water. The experiment was blocked by time with one block, consisting of one slide of each of the eleven treatments, being set up and assessed per day. There were three blocks. The treatments were set up and assessed in sequential order for the first block and random for subsequent blocks.

A petroleum jelly B.P. (Boots, Nottingham, England) ring was made by smearing the compound around the edge of a 19mm cover slip and placing it on the slide. The cover slip was removed to leave a shallow ring of petroleum jelly on the slide. Spores were tapped onto the glass microscope slides. Three drops of solution from a pipette were placed in each ring which was then sealed with a glass cover slip. A second seal around each cover slip was made using nail varnish. The width and length of the spores were measured using an eye piece graticule calibrated with a stage micrometer. All measurements and assessments for plasmolysis and germination were performed under a 400x magnification. Plasmolysis was defined as a visible separation of the cell membrane from the cell wall and germination as the production of a secondary germ tube from the spore which had a length twice its width. This germ tube was the germ tube which gives rise to an infection peg and was not confused with the primary recognition germ tube. A minimum of three randomly selected fields and 30 spores in total on each slide were assessed at 3 hourly intervals for 12 hours. Spore length was divided by spore width to

give a circularity index. The rationale for this was to identify changes in spore shape, expressed as a ratio of length to width, in a numerical fashion. The advantage of this index over separate measurements of length and width were that it was independent of differences in actual spore size and could cope with simultaneous changes in length and width in one step.

The data for all variables were analysed using analysis of variance with polynomial contrasts over osmotic potential. The spore germination and plasmolysis data satisfied the assumptions of the binomial distribution. For the analysis of variance the data did not satisfy the assumptions of normality and homogeneity of variance. Therefore the data were transformed using the angular transformation (Mead and Curnow, 1983). As the sample size was less than fifty, a zero proportion was counted as 1/(4n) and one hundred percent as (n-1/4)/n before transforming to angles following the advice of Bartlett (1949) to improve the equality of variance in the angles at the extremes of the ranges.

7.3 Results

Analysis did not show any consistent differences in effect between polyethylene glycol and potassium chloride. It appeared that all patterns in the differences of response to osmotic potential were essentially evident after six hours and therefore only data from this assessment are illustrated. The data for length, width, percentage germination and plasmolysis are shown plotted as a function of osmotic potential (Figures 13,14,15,16 and 17). The data for this and other assessment times are shown in Table 7.1.

Spore (Figure 13) length was reduced by osmotic potential with a strong linear trend (P=0.01) which was evident at all assessments. A source effect was indicated after twelve hours but this did not fit the trend produced by previous assessments. Water did not appear to cause any change in spore length at any time. There was some evidence of a decline in length with time for concentrations with an osmotic potential exceeding 25.65 bar although this could not be tested statistically due to non-independence of data between times.

At time zero there was a negative linear correlation between osmotic potential and spore width (P=0.05). This trend was evident at all other assessments with a very high significance (P=0.01). This is illustrated for the assessment after six hours in Figure 14. After six hours (Figure 14) a quadratic trend was also present in the data (P=0.05) but was not present at any other time. Therefore its occurrence was regarded as being due to random chance. The initial linear trend in the data was possibly due to the effect of osmotic potential on spore width being a rapidly occurring phenomenon and that the initial assessment at time zero detected effects occurring within the first ten minutes following immersion of the spores in solution. This data is presented in Table 7.1.

The analysis of spore circularity indices at time zero revealed no significant differences between treatments. The index of spores in the water treatment changed by a small amount in relation to the other treatments. A rapid increase in the index

was observed over the initial three hours of the experiment after which the indices stabilised. This data is presented in Table 7.1. After three hours and at all subsequent assessment times a very highly significant (P=0.01) negative linear correlation between index and osmotic potential was observed. This was the same regardless of the source of osmotic potential.

As described in the material and methods the data relating to spore germination and plasmolysis were transformed. However, the procedure for data presentation suggested by Mead and Curnow (1983) was followed. The graphs shown relating to these variables are derived from the untransformed data but tables are given for both untransformed (Table 7.2) and transformed data (Table 7.3).

The percentage germination at time zero or after three hours was not analysed because at this time germination was very low and indeed did not occur in two blocks. At each assessment a strong negative linear trend was found in the data relating to spore germination (P=0.001). This was illustrated for the six hour assessment (Figure 16). Differences between the osmotica were found which were significant (P=0.05) after six and nine hours only and they alternated in effectiveness. This suggested that no real difference existed, particularly as the differences were attributable to a cubic interaction.

At all assessments after time zero plasmolysis was significantly affected by osmotic potential (P>0.001). Plasmolysis increased with osmotic potential in a linear manner (P=0.001) (Figure 17). Plasmolysis was not affected by any factor other than the osmotic potential.

Figure 13 Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the length of *Erysiphe graminis* spores after six hours immersion in solutions. (bar=S.E.M.)

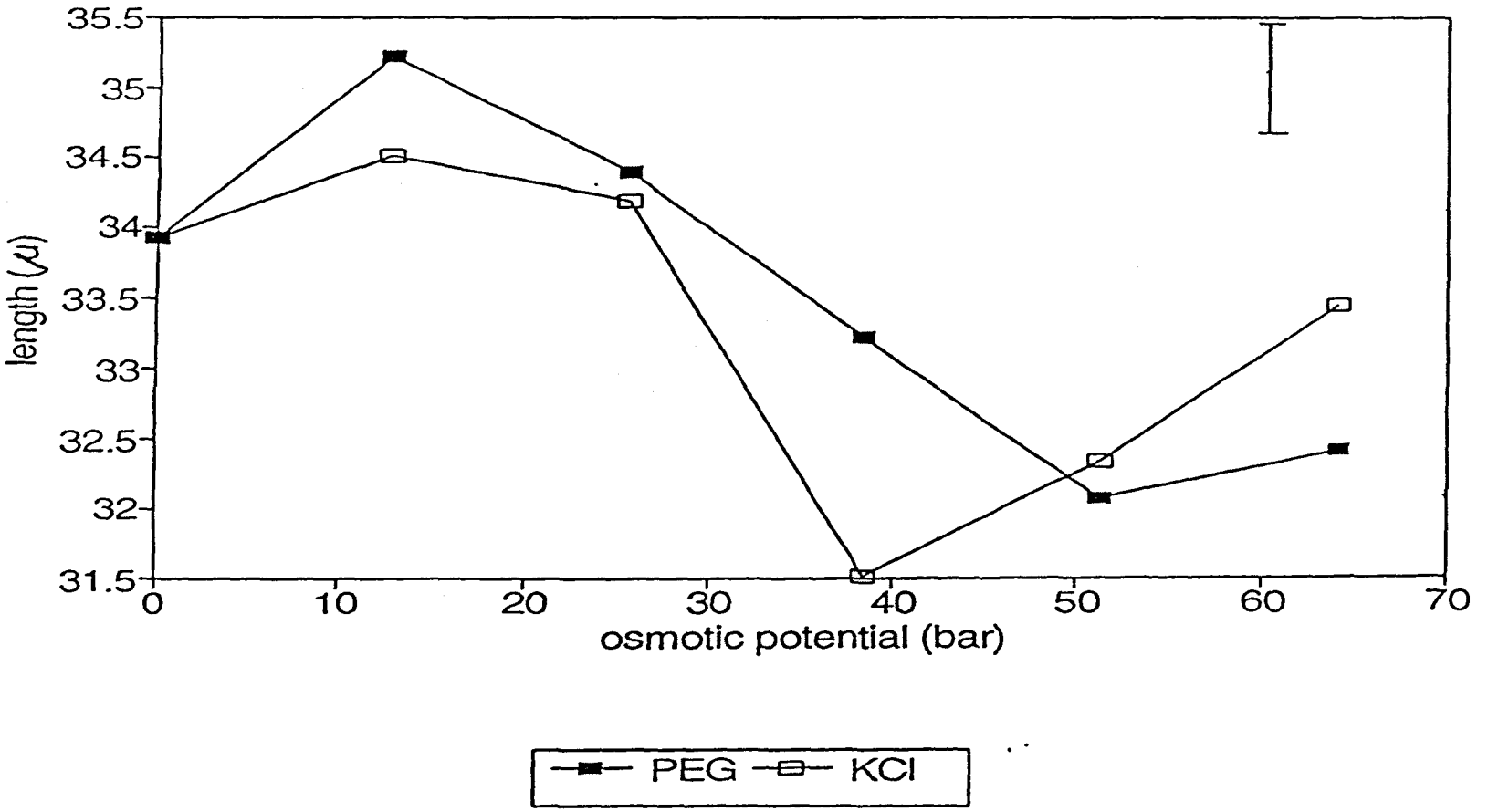


Figure 14 Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the width of *Erysiphe graminis* spores after six hours immersion in solutions. (bar=S.E.M.)

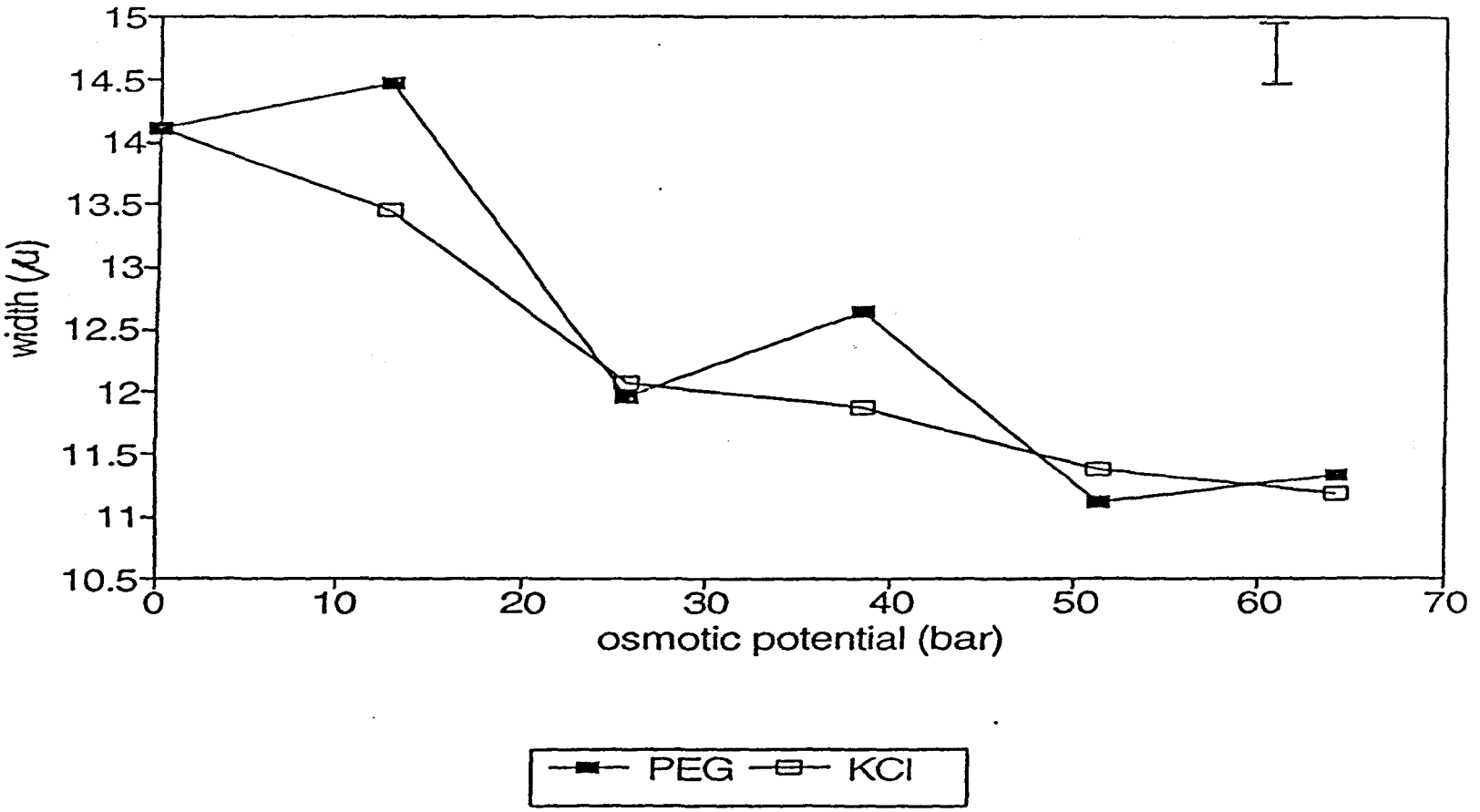


Figure 15 Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the circularity index of *Erysiphe graminis* spores after six hours immersion in solutions.

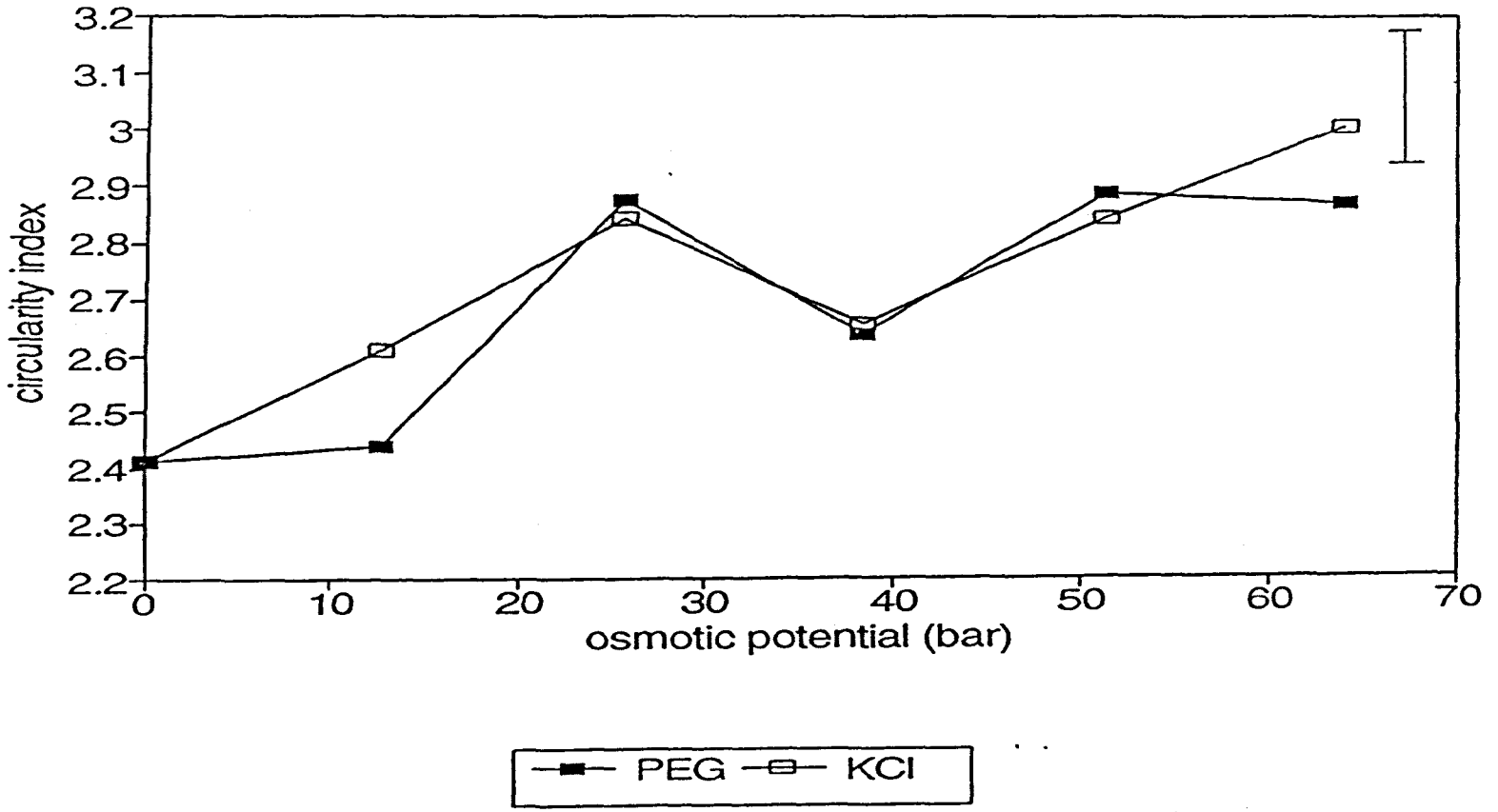


Figure 16 Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the germination of *Erysiphe graminis* spores after six hours immersion in solutions.

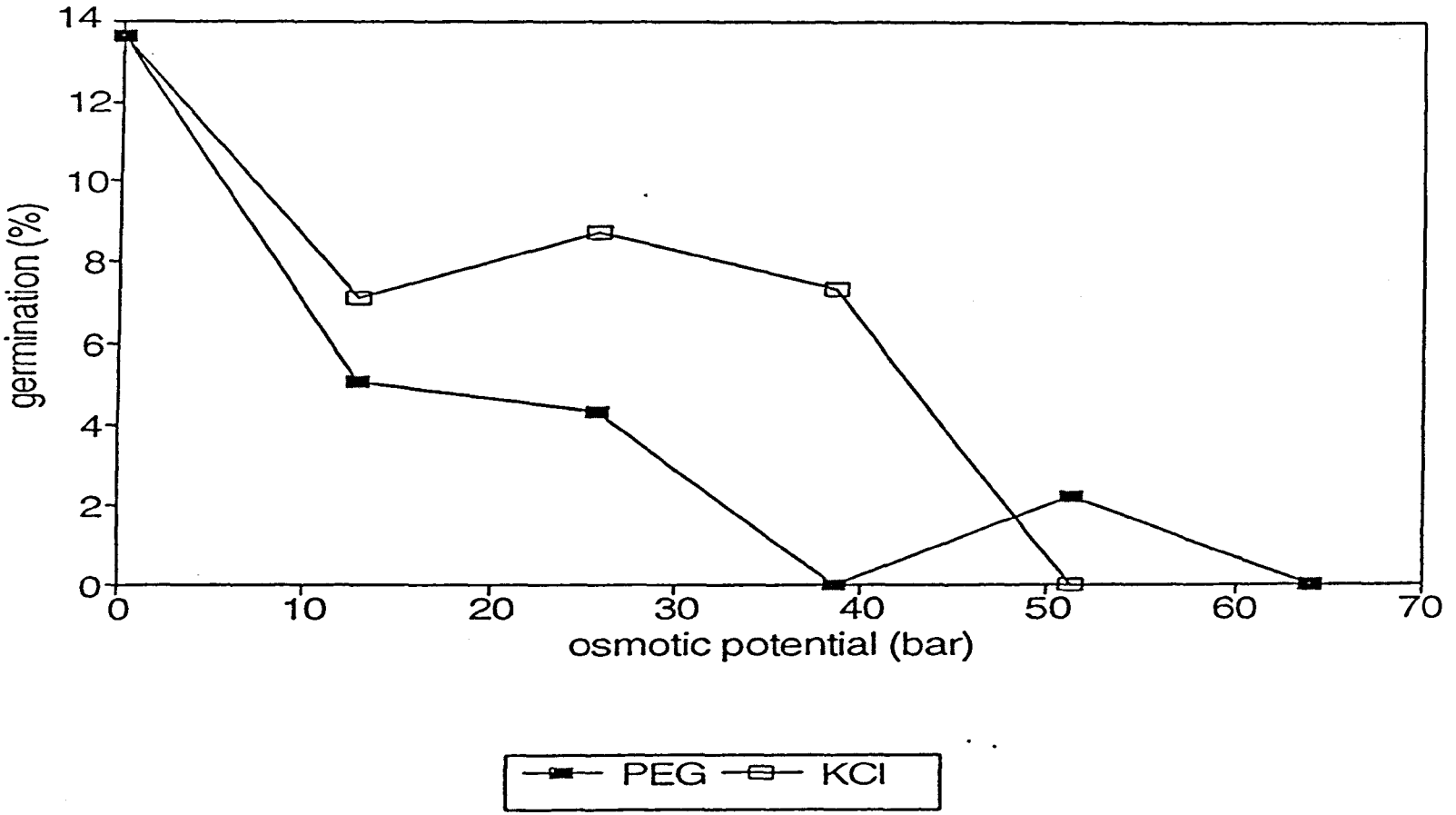


Figure 17 Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the plasmolysis of *Erysiphe graminis* spores after six hours immersion in solution.

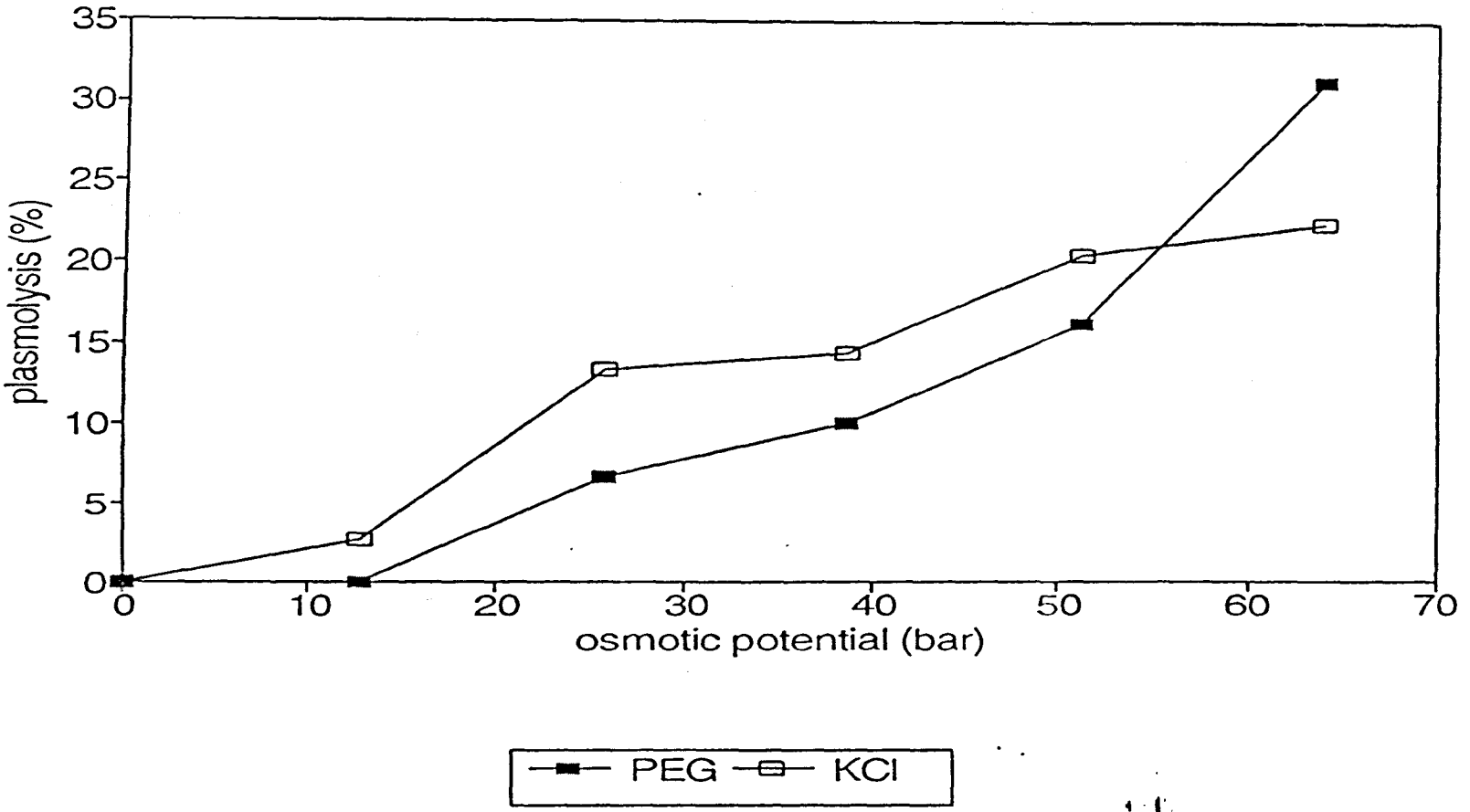


Table 7.1. Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on length, width and circularity of *Erysiphe graminis* spores at the commencement of the experiment and after three, six, nine and twelve hours.

Osmotic potential		0	12.83	25.65	38.48	51.31	64.14	S.E.M.	C.V.%
Time 0 hours									
length mm	KCl	33.78	34.89	34.58	32.49	33.00	33.62	0.749	3.9
	PEG	33.78	33.92	33.77	33.23	32.77	33.94		
width mm	KCl	14.32	14.72	14.30	12.66	13.76	13.55	0.450	5.6
	PEG	14.32	14.43	13.82	13.82	13.50	13.36		
index	KCl	1.09	1.17	1.16	1.25	1.17	1.20	0.0393	5.8
	PEG	1.09	1.13	1.19	1.16	1.17	1.23		
Time 3 hours									
length mm	KCl	33.94	34.82	33.50	33.42	33.60	33.04	0.754	3.9
	PEG	33.94	34.25	34.41	33.26	33.89	33.53		
width mm	KCl	14.01	13.33	12.82	11.76	11.40	11.21	0.520	7.3
	PEG	14.01	13.44	12.73	11.73	11.57	11.49		
index	KCl	2.43	2.63	2.62	2.84	2.96	2.95	0.090	5.7
	PEG	2.43	2.56	2.72	2.84	2.93	2.93		
Time 6 hours									
length mm	KCl	33.93	34.85	34.19	31.51	32.34	33.45	0.890	4.6
	PEG	33.93	35.21	34.42	33.22	32.07	32.41		
width mm	KCl	14.11	13.45	12.06	11.87	11.37	11.17	0.400	5.6
	PEG	14.11	14.47	11.96	12.65	11.11	11.33		
index	KCl	2.41	2.61	2.84	2.66	2.84	3.01	0.0931	5.9
	PEG	2.41	2.44	2.88	2.64	2.89	2.87		
Time 9 hours									
length mm	KCl	34.43	32.85	33.29	32.54	32.26	32.70	0.618	3.2
	PEG	34.43	33.39	34.60	33.04	31.63	32.72		
width mm	KCl	14.29	13.87	12.20	12.40	11.29	11.30	0.510	7.1
	PEG	14.29	14.00	12.84	12.08	11.16	11.74		
index	KCl	2.35	2.39	2.73	2.63	2.87	2.90	0.1119	7.3
	PEG	2.35	2.40	2.70	2.74	2.83	2.81		
Time 12 hours									
length mm	KCl	34.29	33.59	32.83	30.84	32.43	31.80	0.6962	3.7
	PEG	34.29	33.05	34.54	33.16	32.05	33.41		
width mm	KCl	17.07	13.11	11.84	11.65	12.23	10.29	0.0206	14.1
	PEG	17.07	13.65	13.08	12.06	12.10	10.53		
index	KCl	2.10	2.56	2.78	2.65	2.70	3.10	0.149	9.6
	PEG	2.10	2.46	2.65	2.78	2.68	3.20		

KCl = potassium chloride PEG = polyethylene glycol
d.f.= 20

Table 7.2. Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the percentage of *E. graminis* spores germinated and plasmolysed at the commencement of the experiment and after three, six, nine and twelve hours (untransformed data).

Osmotic potential(bar)		0	12.83	25.65	38.48	51.31	64.14
Time 0 hours							
plasmolysis%	KCl	0.0	0.0	0.0	0.0	0.0	0.0
	PEG	0.00	0.0	0.0	0.0	0.0	0.0
germination%	KCl	0.00	0.0	0.0	0.0	0.0	0.0
	PEG	0.00	0.0	0.0	0.0	0.0	0.0
Time 3 hours							
plasmolysis%	KCl	0	0	5.56	3.61	11.8	17.3
	PEG	0	0	1.44	7.78	6.0	9.2
germination%	KCl	3.8	6.7	0.0	4.0	3.8	0.0
	PEG	3.8	0.0	2.02	0.0	0.0	0.0
Time 6 hours							
plasmolysis%	KCl	0	2.7	13.4	14.0	20.5	22.3
	PEG	0	1.3	6.9	9.1	17.7	16.8
germination%	KCl	13.6	7.2	8.8	7.3	0	8.9
	PEG	13.6	5.1	4.3	0	2.2	0
Time 9 hours							
plasmolysis%	KCl	1.0	10.4	0.0	4.0	4.3	0.0
	PEG	1.0	13.6	6.6	4.2	0.0	0.0
germination%	KCl	0	0	23.8	12.8	36.7	39.2
	PEG	0.0	4.2	13.3	22.7	33.7	37.7
Time 12 hours							
plasmolysis%	KCl	0	1.4	21.0	32.8	40.1	54.4
	PEG	0	2.4	21.1	17.6	38.7	50.4
germination%	KCl	16.5	14.7	4.2	0.0	4.3	0
	PEG	16.5	12.0	2.2	0.0	2.0	0.0

KCl = potassium chloride PEG = polyethylene glycol

d.f.= 20

Table 7.3. Experiment SO1. The effect of osmotic potential created with potassium chloride or polyethylene glycol on the percentage of *Erysiphe graminis* spores germinated and plasmolysed at the commencement of the experiment and after three, six, nine and twelve hours (angular transformed data).

osmotic potential		0	12.83	25.65	38.48	51.31	64.14	S.E.M.	C.V.%
Time 3 hours									
plasmolysis	KCl	0.009	0.009	0.283	0.268	0.337	0.433	0.663	54.4
	PEG	0.009	0.009	0.077	0.333	0.226	0.314		
Time 6 hours									
plasmolysis	KCl	0.009	0.137	0.405	0.466	0.472	0.524	0.0638	35.8
	PEG	0.009	0.039	0.261	0.282	0.350	0.455		
germination%	KCl	0.352	0.224	0.317	0.311	0.009	0.009	0.0477	51.9
	PEG	0.352	0.297	0.149	0.009	0.067	0.009		
Time 9 hours									
plasmolysis	KCl	0.009	0.009	0.453	0.412	0.626	0.747	0.0898	38.8
	PEG	0.009	0.064	0.300	0.437	0.597	0.757		
germination%	KCl	14.32	14.72	14.30	12.66	13.76	13.55	0.450	5.6
	PEG	14.32	14.43	13.82	13.82	13.50	13.36		
Time 12 hours									
plasmolysis	KCl	0.009	0.094	0.494	0.589	0.676	0.985	0.097	34.1
	PEG	0.009	0.152	0.393	0.459	0.606	0.932		
germination%	KCl	14.32	14.72	14.30	12.66	13.76	13.55	0.450	5.6
	PEG	14.32	14.43	13.82	13.82	13.50	13.36		

KCl=potassium chloride PEG= polyethylene glycol

d.f.=20

n.b. data for germination at 3 hours not analysed due to very low values causing deviation from normality.

7.4 Discussion

The results obtained differed from some of those obtained elsewhere. The spores did not collapse in distilled water but retained their integrity as described elsewhere (Corner, 1935; Manners and Housain, 1963). A proportion of the spores also retained their viability and produced germ tubes.

It was apparent that the effects observed were due almost entirely to the osmotic activity of the solutions and not the source of the osmotica. Therefore it would be reasonable to expect any salt solution to have the same effects as potassium chloride.

It appeared that increasing the osmotic potential of a solution in contact with the spores resulted in a change in spore shape. The spores became elongated, largely as a result of a decrease in spore width. This was indicated by the increase in the circularity index. Spore width was more affected than spore length, possibly due to directional differences in the strength of the cell wall. The cause of these changes in shape was probably due to an out-flow of water from the spores due to the creation of an osmotic gradient. At high osmotic potentials the effect was rapid and resulted in widespread plasmolysis. This finding was in agreement with the findings of Weeds *et al.* (1993). The plasmolysis was probably the explanation for the reduced germination of spores in solutions of high osmotic potential. These changes were apparent within three to six hours of immersion and therefore would have occurred quickly enough to impair the infection process *in vivo*. Overall it appeared that the critical factor affecting spore germination was the osmotic potential of the solutions rather than the osmotica.

Since the wax cuticle interacts with surface solutions it was difficult to draw direct comparisons between solutions of the same concentration on the leaf surface and *in vitro* as the water potential will be different. Also spray deposition on the leaf surface is uneven, and spores may be affected differentially depending upon their position relative to spray droplets. However, the similarities in the response of *E.*

graminis to potassium chloride *in vitro* and *in vivo* imply that osmotic potential played an important part in the inhibition of *E. graminis* on wheat leaves by potassium chloride and this phenomenon may, at least in part, be responsible for the fungicidal effects of potassium chloride observed in the field.

Chapter 8 - Experiments Investigating the Effects of Solutions of Different Osmotic Potential on Spore Germination, Infection Processes and the Leaf Area of Winter Wheat Affected by *Erysiphe graminis*(Experiment GH5).

8.0 Introduction

The *in vitro* experiments described in Chapter 5 indicated that the leaf area affected by *E. graminis* decreased as the concentration of potassium chloride solution applied, at a fixed volume, was increased. The *in vitro* experiment described in Chapter 7 examining the effect of solution osmotic potential on the spores of *E. graminis* indicated that the germination percentage was reduced by exposure to solutions of high osmotic potential. This response was linearly related to the osmotic potential of the solutions used. The lowering of the germination percentage was paralleled by deformation of the spores and an increase in the percentage of cells suffering plasmolysis. . These factors, coupled with an absence of consistent differences between osmotica at the same osmotic potential, suggested that spore viability was reduced by exposure to solutions of high osmotic potential.

It was thought that the reduction in the leaf area affected by *E. graminis* in response to the application of potassium chloride solution as a foliar spray was due to osmotic activity. This theory was supported by the work of Weeds *et al.* (1993) who showed that the hyphae of cucumber powdery mildew (*Sphaerotheca fuliginea*) were severely damaged by the application of foliar sprays of potassium chloride and other salt solutions. It was suggested that the application of solutions of high osmotic potential may have caused a net outflow of water from the hyphae resulting in their collapse (Weeds *et al.* 1993). Following these observations it was considered that

the activity of potassium chloride against *E. graminis* might be, at least partly, due to an osmotic effect on the fungal cells.

Two possible mechanisms were considered likely. The activity of a solution of high osmotic potential on the leaf surface might inhibit the germination of spores as in the *in vitro* experiment and also inhibit the development of germ tubes by subjecting the hyphae to osmotic stress. The other mechanism considered likely was the osmotic potential of host epidermal cells being altered following the uptake of ions or molecules applied to the leaf surface. If the osmotic potential of the epidermal cell sap was altered in this way it was possible that the establishment of a biotrophic relationship might be inhibited.

At this point in the investigation it was unclear whether the reductions of wheat leaf area affected by powdery mildew observed earlier were due to the osmotic potential of the solution or some other property of solutions created with potassium chloride. It was therefore decided to investigate the effect of solutions, with different osmotica and different osmotic potential, applied to the leaf surface on the leaf area affected by *E. graminis* and the infection process of this pathogen.

8.1 Objectives

1) To investigate the effect of solutions of different osmotic potential on the leaf area affected by *E. graminis*.
2) To investigate the effect of different osmotica on the leaf area affected *E. graminis*.
3) To investigate the effect of solutions of different osmotic potential applied to the leaf on the infection process of *E. graminis*.

4) To investigate the effect of different osmotica applied to the leaf on the infection processes of *E. graminis*.

8.2 Materials and Methods

The experiment was conducted using unvernalised wheat plants (cv. Apollo) at the three fully expanded leaf stage. The plants were grown in low potassium compost from pre-chitted seed as described in general materials and methods and selected forty five days after sowing in FP7 pots (Plantpak, Maldon, England). The plants were grown in the spore free propagator with an ambient air source. The plants were inoculated using spores from stock pots containing the wheat cultivar Apollo produced as described in general materials and methods. The stock pots were inoculated twelve days before the experiment and maintained at a minimum temperature of 15°C by day and 5°C by night on gravel beds which were regularly wetted to maintain humidity. Plants were removed from the propagator at the commencement of the experiment and selected for uniformity. After selection plants were allocated to treatments using random numbers.

The experiment was of a randomised block design with two factors: osmotica at two levels, and osmotic potential of solution at six levels. Treatments were a control sprayed with pure deionised distilled water, an untreated control and solutions of 12.83, 25.65, 38.48, 51.31 and 64.14 bar osmotic potential made up using either potassium chloride or polyethylene glycol (PEG). These osmotic potentials were equivalent to 2,3,6,8 and 10% w/v potassium chloride and 10.76, 21.45, 32.17, 42.90 and 53.62% w/v PEG 200 solutions (BDH, Poole, England). All solutions were made up using sterile distilled water.

The experiment consisted of two parts, a randomised block design with twenty five replicates taken through to pustule emergence and a ten block randomised design which was destructively sampled twenty four hours after inoculation. The plants, except the unsprayed control, were sprayed using a precision pot sprayer fitted with a medium flat fan jet (015-F80, Lurmark, Cambridge, England) at approximately 3 bar and a rate equivalent to 200l/ha. The precision pot sprayer was a purpose built experimental sprayer with a moving spray head which travelled along a fixed gantry. The application rate was calibrated by measuring the flow rate of the spray head and using tables to convert the value to volume per unit area.

After treatment the plants were set out on a glasshouse bench over damp gravel in randomised blocks (Plate 10). The pots in each block were laid out in a four by three grid pattern. The blocks were arranged as a five by five grid with an adjacent two by five grid for destructive sampling. After three hours the leaf surface bore no visible water and the experiment was inoculated by shaking heavily infected stock pots over the plants. The stock pots had been shaken the previous night to remove any spore chains.

After twenty four hours the destructive sampling of ten blocks was carried out. The upper fully expanded leaf of each plant was detached by cutting at the ligule and placed in the leaf clearing apparatus.

The leaf clearing apparatus consisted of a plastic tray with a four millimetre thick glass sheet laid in the bottom. A blotting paper sheet was laid over this sheet as a wick (Plate 11). Leaves were laid on the wick and the solvents were added to the

wick by pouring them into the tray. The whole apparatus was sealed in a plastic bag. Wicks were changed daily (Plate 12).

The clearing method followed one described by Smith (pers. com). For the first stage, 72 hours, the solvent was a 3:1 by volume mixture of absolute ethanol and acetic acid. When the leaf was cleared the wick was soaked with 95% ethanol, 90% ethanol and sterile distilled water for twenty four hours in sequence. The leaves were then stored in petri dishes on pads of lactoglycerol prepared by mixing lactic acid, glycerol and water in equal parts by volume. The upper side of the third leaf was then examined under a microscope following staining with aqueous trypan blue (0.1% w/v). All of the leaf was observed. Counts were made of the number of spores per leaf. The spores were categorised into classes of ungerminated, primary germ tube produced, secondary germ tube produced, appressoria produced and infection peg produced. The percentages of germinated spores and spores which had produced an infection peg were calculated and subjected to angular transformation to improve the homogeneity of variance and normality of these binomially distributed data before analysis. Following Mead and Curnow (1983) graphs are presented untransformed without standard error bars and tables are presented showing both transformed and untransformed data.

After two weeks the remaining plants were assessed for percentage area of the third and fourth fully expanded leaves from the stem base affected by *E. graminis* using visual inspection aided by ADAS disease assessment keys (Anon, 1976) following practice using computer training aids. These were the upper two fully expanded leaves at the time of inoculation. Both the upper and lower leaf surfaces were assessed. A small number of plants retained in the spore free propagation unit

Plate 10 Experiment GH5 *in situ* on a glasshouse bench immediately after inoculation.

Plate II The leaf clearing apparatus two days after commencement with the cover removed for unobstructed view.

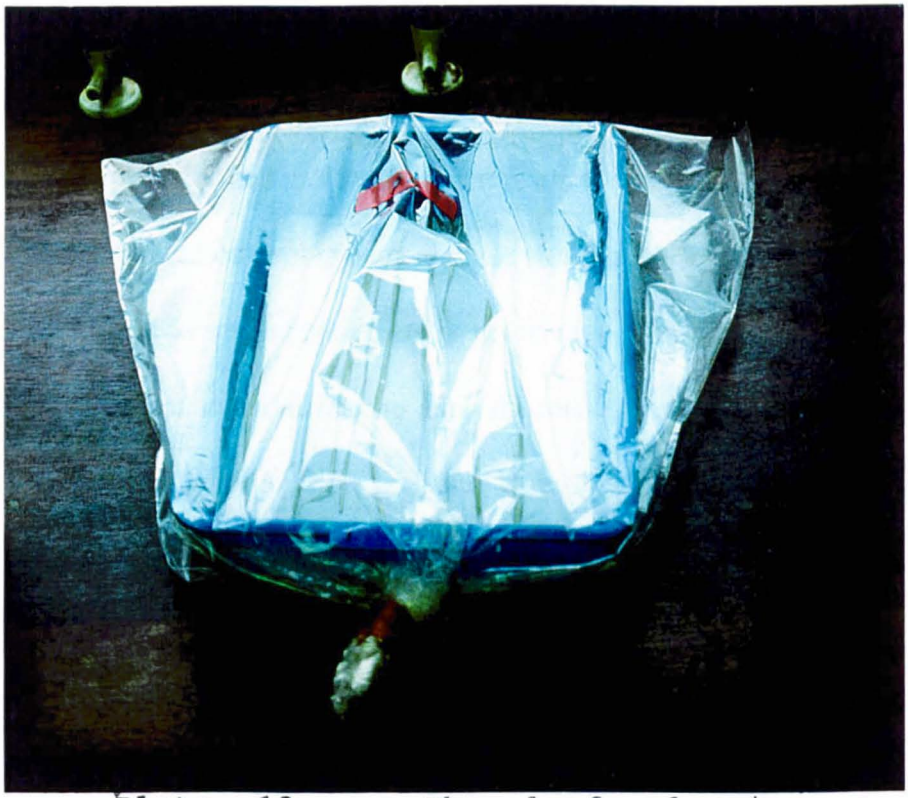


Plate 12 The leaf clearing apparatus shown complete and enclosed in a plastic bag.

were inspected to ensure that the plants had been free of disease at the start of the experiment. The results were analysed by a blocked factorial analysis of variance with polynomial contrasts (Ridgeman,1975).

It was thought that inhibition of spore germination after twenty four hours might be related to the severity of visual disease symptoms. To test this it was essential that both sets of data were on the same scale. To do this the mean leaf areas affected and transformed percentage germination for each osmotic potential were used. The untreated control in each data set was given a value of one hundred and all the other values were expressed as a percentage of this. The zero bar (water) treatment was not included in the analysis and it was common to both levels of osmotica. A parallel regression was then carried out on the data sets with osmotic potential as the independent variable. This enabled the comparison of the two lines.

8.3 Results

The area of all leaves, both upper and lower surfaces, affected by *E. graminis* declined as the osmotic potential of the solutions increased (Figures 18, 19, 20 and 21). The decline was linear (P=0.001) and there was no significant difference between the two sources of osmotic potential on either leaf at the P=0.05 level of significance. The absence of any significant deviations indicated that the reductions in leaf area affected by *E. graminis* were solely attributable to variations in the osmotic potential of the solutions. A difference between the untreated control and the water sprayed control treatments revealed that the application of water alone reduced leaf area affected (Appendix B4).

The microscopic examination revealed that some spores could germinate, defined as the production of a secondary germ tube, under all treatments. However the germination percentage declined in a linear manner with increasing osmotic potential of the solution applied (P=0.001) (Table 8.1). This was clearly apparent when plotted (Figure 22). A similar decline occurred for the percentage of spores germinating and forming infection pegs (Table 8.1). There was no significant difference between the two osmotica.

The parallel regression of the mean leaf area affected and percentage germination of the *E. graminis* spores on the upperside of the fourth leaf after conversion to the same scale against osmotic potential of the solutions applied yielded significant results. The overall regression was significant (P=0.001) indicating that both data sets had strong linear trends (Figure 23). The slope of the curve for leaf area affected, -1.0392, was not significantly different from that for spore germination, -0.778844, although the position of the two lines was significantly different (P=0.05) (Appendix B6). Very few spores were seen at an intermediate stage of development between a non-germinated state and infection peg formation. It was possible to discern, under high magnification, that some of the ungerminated spores had undergone plasmolysis. Photo-micrographs were taken on a Leitz DMR microscope fitted with a Wild MPS 48 photo system supplied by Leica using Fuji ASA 200 colour print film using automatic exposure control following the manufacturer's standard operating procedure.

These micro-graphs clearly illustrated that *E. graminis* spores could germinate and produce infection pegs under a range of treatments (Plates 13 and 14). Micro-graphs of ungerminated spores following the application of a 12.83 (Plate 15)

and a 51.31 bar solution (Plate 16) to the leaf surface illustrate the shrinking of the spores with a marked decrease in width. It was difficult to get clear pictures of the spores due to problems with the depth of focus required.

Figure 18. Experiment GH5. The effect of solutions of different osmotic potential created with two osmotica, potassium chloride or polyethylene glycol, on the area of the fourth leaf from the stem base (lower side) of wheat affected by *E. graminis*. (bar = S.E.M.)

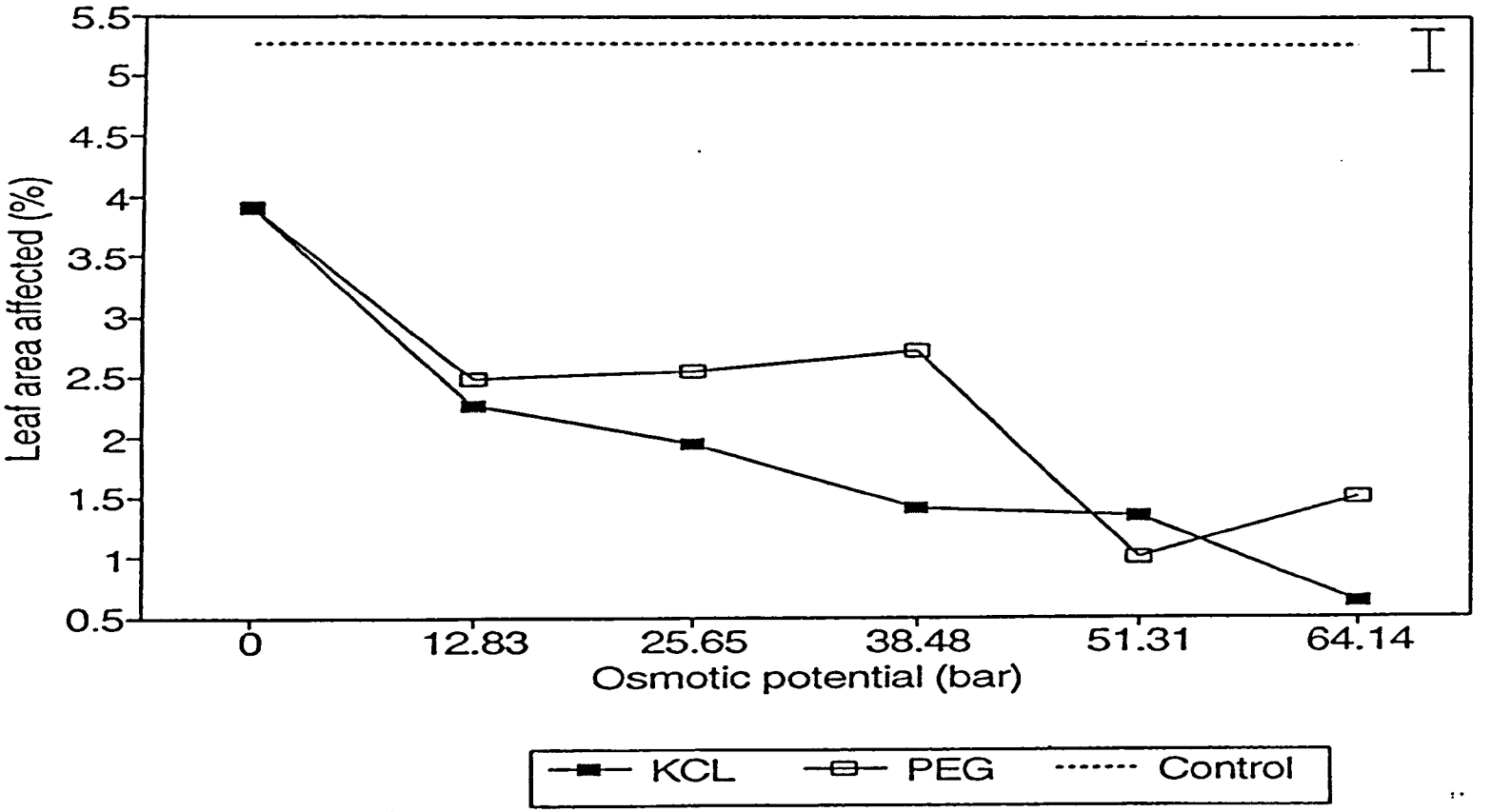


Figure 19. Experiment GH5. The effect of solutions of different osmotic potential created with two osmotica, potassium chloride or polyethylene glycol, on the area of the fourth leaf from the stem base (upper side) of wheat affected by *E. graminis.* (bar = S.E.M.)

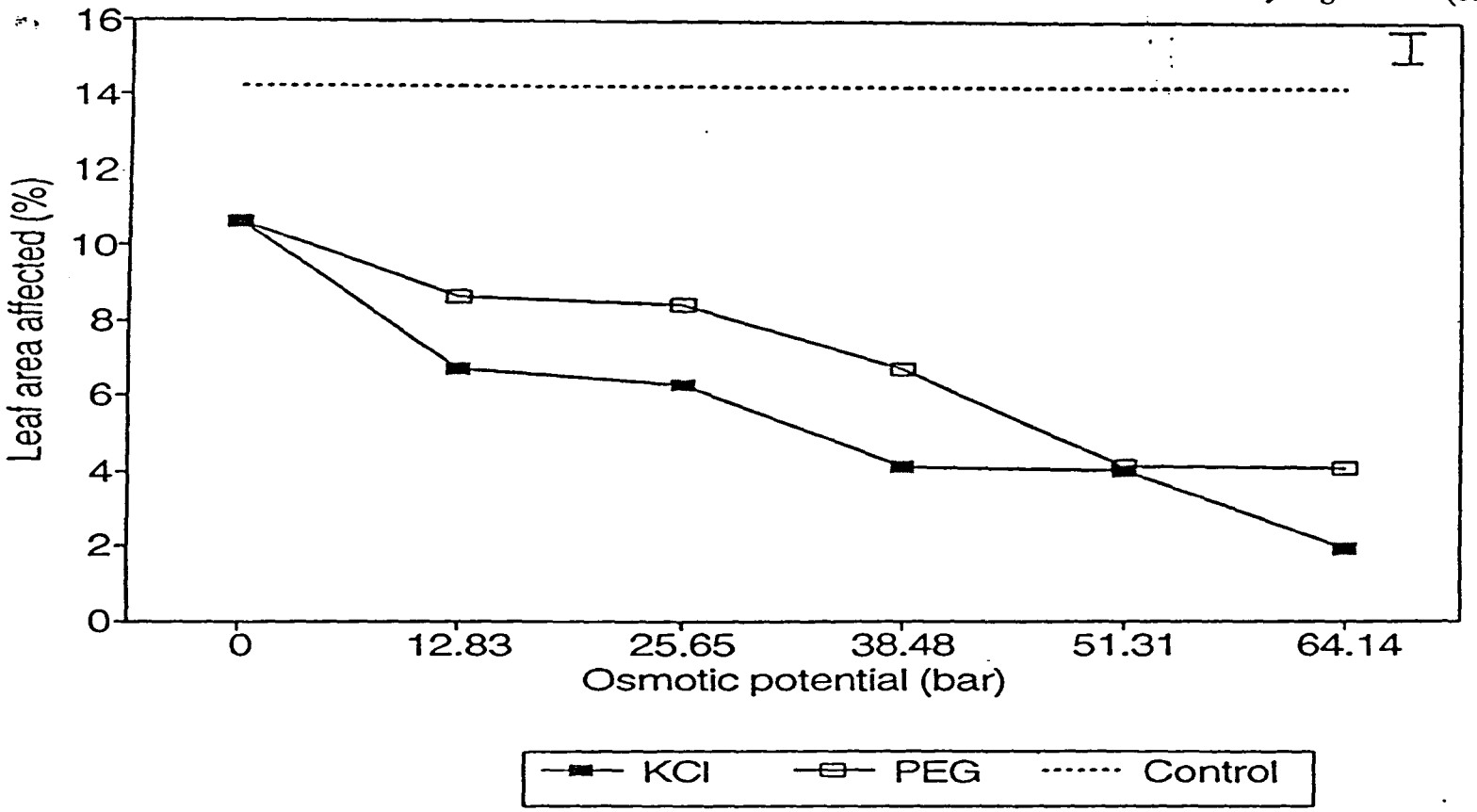


Figure 20. Experiment GH5. The effect of solutions of different osmotic potential created with two osmotica, potassium chloride or polyethylene glycol, on the percentage area of the third leaf (lower side) from the stem base of wheat affected by *E. graminis*. (bar = S.E.M.)

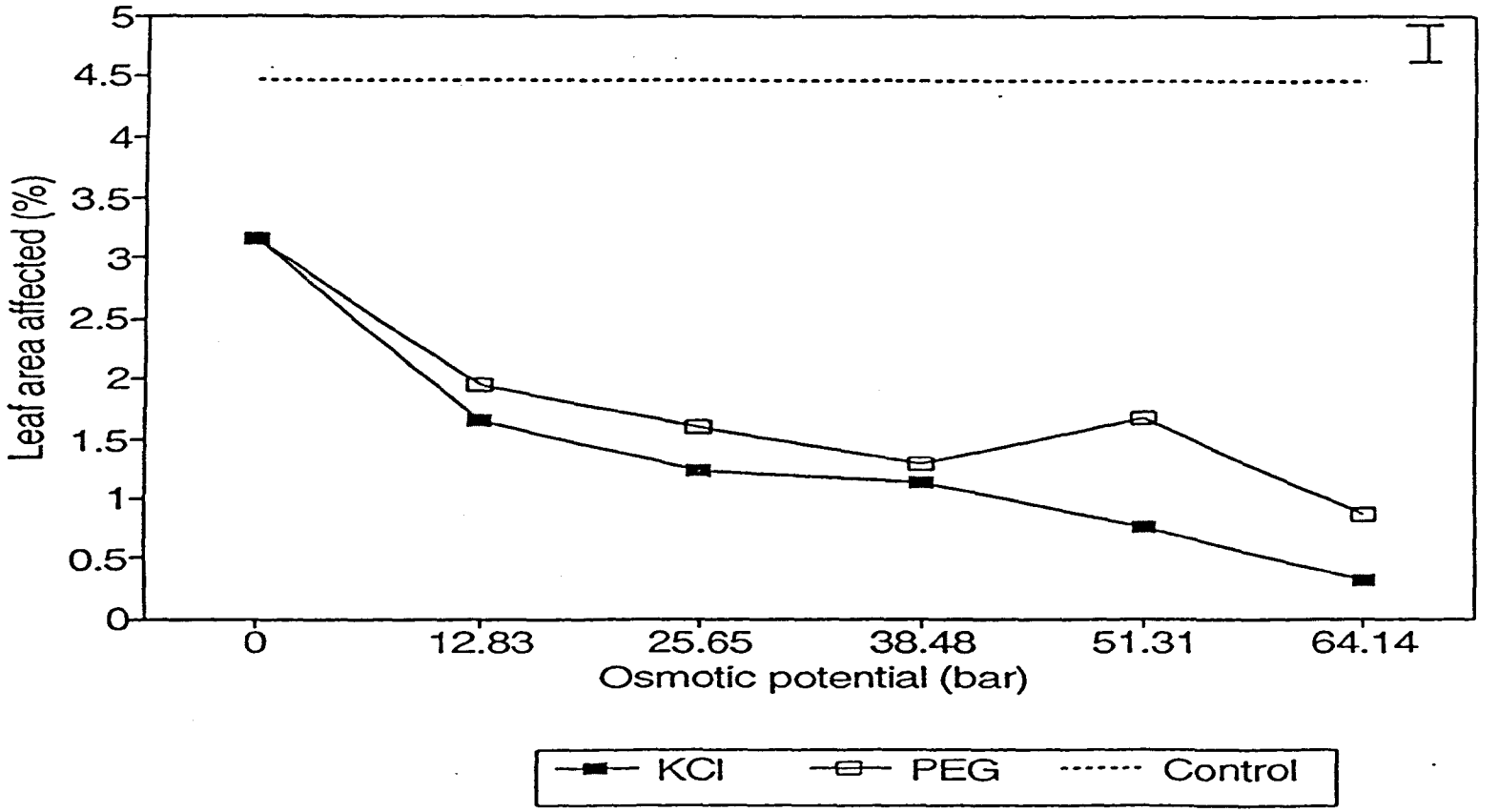


Figure 21. Experiment GH5. The effect of solutions of different osmotic potential created with two osmotica, potassium chloride or polyethylene glycol, on the percentage area of the third leaf (upper side) from the stem base of wheat affected by *E. graminis*. (bar = S.E.M.)

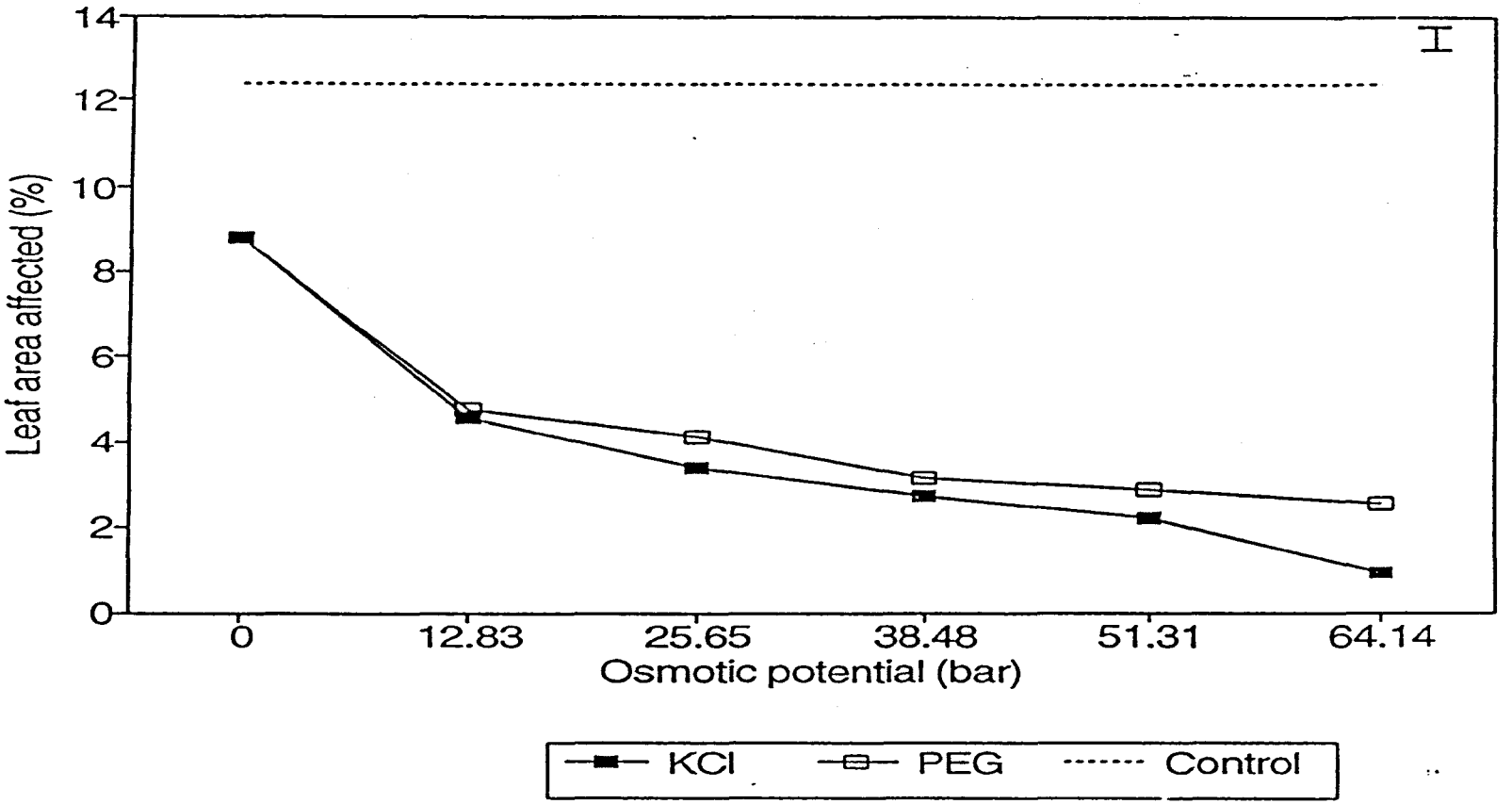


Figure 22. Experiment GH5. The effect after twenty four hours of solutions of different osmotic potential created with two osmotica, polyethylene glycol and potassium chloride, on the percentage germination of *Erysiphe graminis* spores on the upper surface of the fourth leaf from the stem base of winter wheat cv. Apollo.

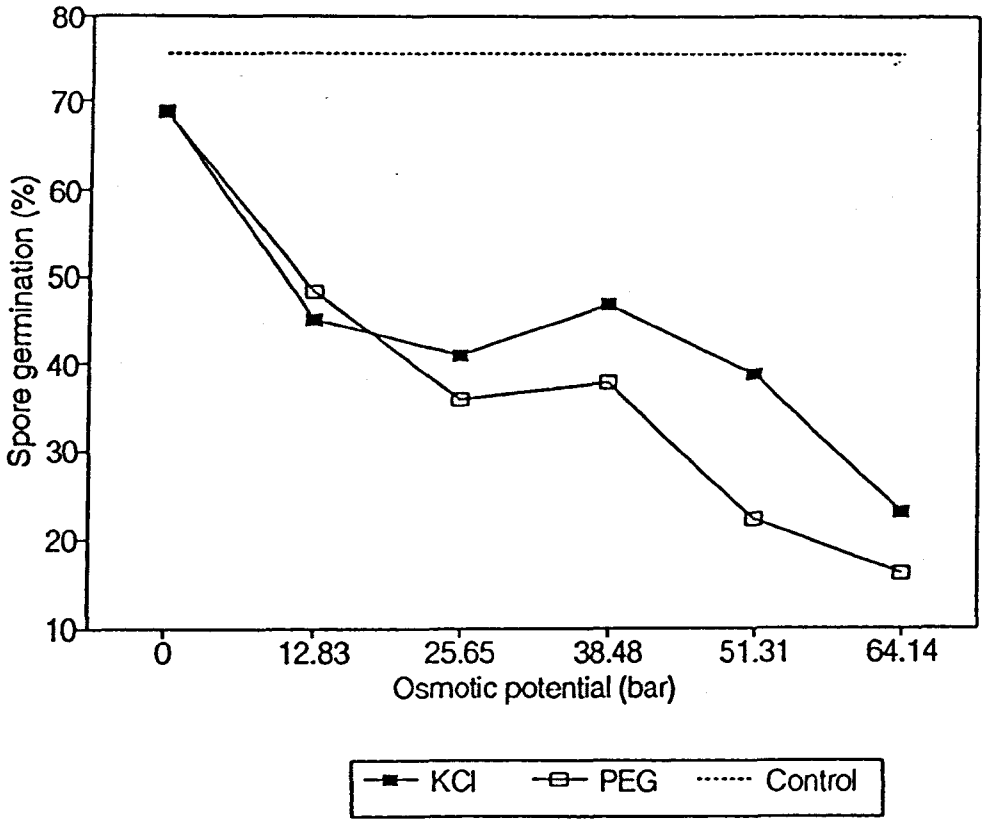


Table 8.1. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the percentage of *E. graminis* spores germinating and producing infection pegs *in vivo* (Experiment GH5)

	Germination%		Infection Peg%	
	Raw data	Transformed	Raw data	Transformed
Control	75.5	1.071	73.8	1.045
water	68.9	0.987	67.3	0.968
KCl 12.83 bar	45.2	0.741	43.7	0.724
KCl 25.65 bar	41.1	0.694	40.1	0.679
KCl 38.48 bar	46.8	0.748	46.2	0.745
KCl 51.31 bar	38.8	0.657	31.8	0.574
KCl 64.14 bar	23.0	0.444	22.0	0.433
PEG 12.83 bar	48.2	0.726	47.8	0.721
PEG 25.65 bar	36.1	0.631	35.3	0.618
PEG 38.48 bar	37.9	0.639	37.1	0.631
PEG 51.31 bar	22.3	0.476	21.3	0.462
PEG 64.14 bar	16.2	0.338	14.6	0.358

ANOVA

BLOCK	NS	NS
Treatment	***	***
Osmotic	***	***
Linear	***	***
Quadratic	NS	NS
Cubic	NS	NS
Deviations	NS	NS
Source	NS	NS
Osmotic*Source	NS	NS
Linear*Dev	NS	NS
Quadratic*Dev	NS	NS
Cubic*Dev	NS	NS
Deviations	NS	NS

S.E.M.		
Treatments	0.073	0.075
Osmotic	0.236	0.270
Source	0.149	0.171
Osmotic*Source	0.334	0.382

C.V.	33.8	35.9

KCl = Potassium chloride PEG = Polyethylene glycol

Plate 13 A photomicrograph of an *Erysiphe graminis* f.sp. *tritici* spore germinating and forming an appresorium and infection peg on a leaf treated with pure water.

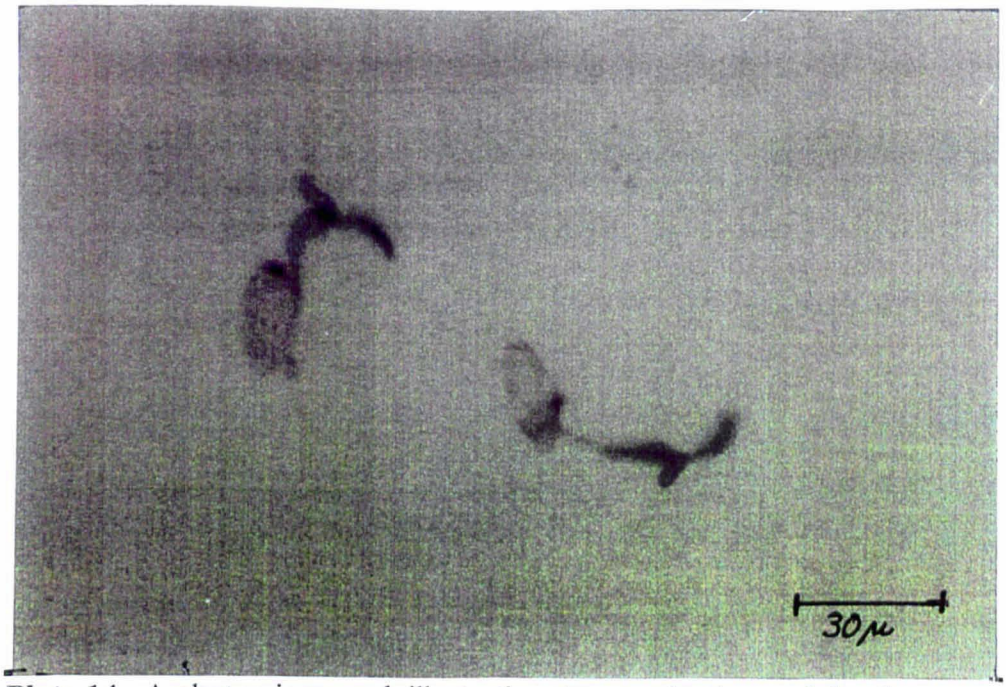


Plate 14 A photomicrograph illustrating the germination and development of an *Erysiphe graminis* f.sp. *tritici* spore on a leaf treated with a 51.31 bar solution of potassium chloride.

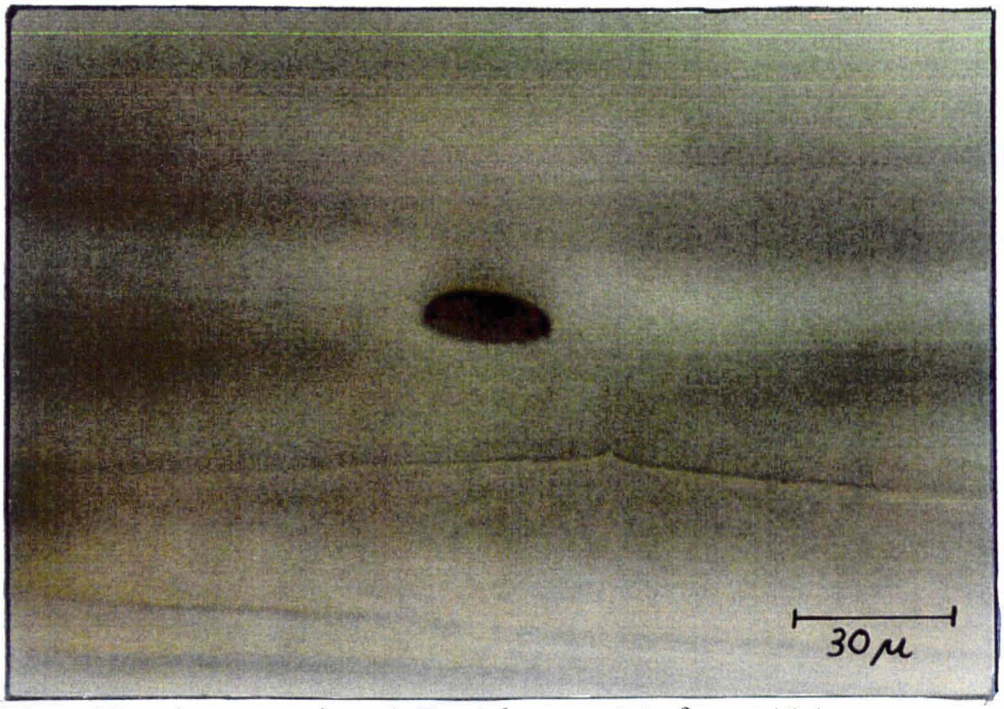
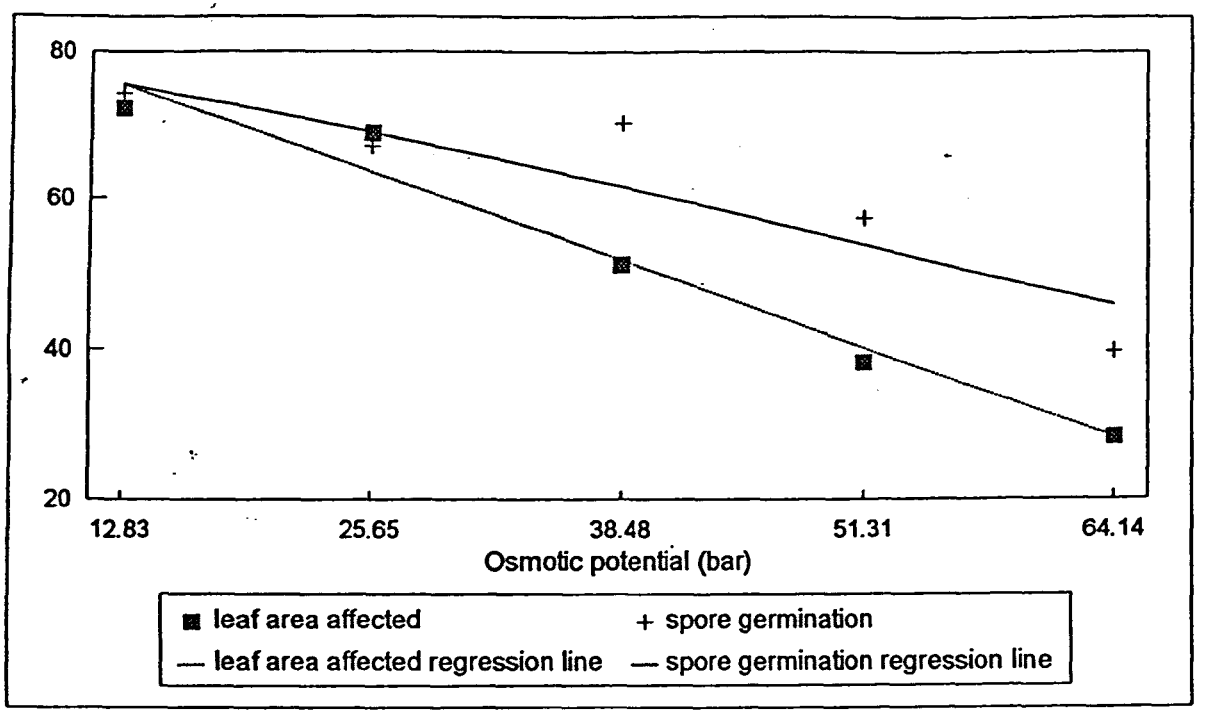


Plate 15 An ungerminated *Erysiphe graminis* f.sp. *tritici* spore on a wheat leaf treated with a 12.83 bar solution of polyethylene glycol.

Plate 16 An ungerminated *Erysiphe graminis* f.sp. *tritici* spore on a wheat leaf treated with a 51.31 bar solution of potassium chloride.

Figure 23. Experiment GH5. The effect of osmotic potential on the percentage leaf area affected by powdery mildew after two weeks and the percentage germination of *Erysiphe graminis* spores after twenty four hours. Data taken from the fourth leaf from the stem base of unvernalised wheat cv. Apollo. Osmotic potential was generated with two osmotica, polyethylene glycol or potassium chloride, and the results averaged. Data expressed as a percentage of the results for zero bar (pure water) treatments.



8.4. Discussion

It appeared that a critical factor in the reduction of leaf area affected by powdery mildew and the percentage germination of *E. graminis spores* was the osmotic potential of the solution applied to the leaf. In neither case was the osmoticum a significant factor affecting the results. In both cases the decline was in a linear manner and it was tempting to suggest a causal relationship between the changes in spore germination and percentage leaf area affected by powdery mildew. This was further encouraged by the parallel regression of the percentage leaf area affected and percentage spore germination against solution osmotic potential.

The relationship between the two curves was surprisingly good as only a few surviving propagules would be expected to result in quite large areas of the leaf exhibiting disease symptoms. The fact that large numbers of germinating spores still failed to produce severe disease symptoms on leaves receiving the more concentrated solutions suggested that some other mechanism might inhibit the establishment of an infection by any germinating spores.

It was suspected that the reduction in germination was due to the plasmolysis of the spores. This was observed to be more severe as the osmotic potential was increased. The survival of large numbers of spores on the leaves of plants treated with solutions of high osmotic potential could be explained by two possible mechanisms. It was possible that moisture on the leaf surface diluted the solutions applied, in local areas, rendering them less effective or the application of the droplets to the leaf surface left some areas uncovered. Following Matthews (1979) the latter hypothesis was considered the more likely, particularly when using a medium droplet spray.

It was concluded that the inhibition of spore germination by solutions of high osmotic potential was a major, but not the only, mechanism explaining the reduction of leaf area affected by *E. graminis* when potassium chloride was applied to cereals as a foliar spray.

Chapter 9 - The Effect of Osmotically Active Solutions on the Development of *Erysiphe graminis in vivo* (Experiment GH6).

9.0 Introduction

In the previous experiment very few propagules were seen at an intermediate stage of development between a non-germinated state and infection peg formation. It was assumed that these developed propagules would go on and form an infection. On control treatments it was apparent that many did. However on leaves treated with highly concentrated solutions of potassium chloride and polyethylene glycol this development was not manifested as visible colonies several weeks after inoculation. This suggested that the spore forming an infection peg did not go on to form a haustoria and a successful infection. An experiment was conducted to investigate this.

9.1 Objective

To evaluate the effect of applying osmotically active solutions on the development of *Erysiphe graminis* on wheat leaves with particular reference to haustorial formation.

9.2 Materials and Methods

Wheat plants, cv. Apollo, were grown in a spore free propagator in low potassium compost as described in general materials and methods. The plants

were selected for uniformity at the four leaf stage and placed on a glasshouse bench.

The experiment was a randomised block design with four treatments and ten replicates. Treatments were foliar sprays of potassium chloride and polyethylene glycol (both 64.4 bar) solutions, a foliar spray of water and an untreated control.

All foliar sprays were applied at 210 l/ha at approximately 3 bar pressure using a precision pot sprayer as described in general materials and methods. The plants were allowed to stand on the bench until touch dry before being inoculated by shaking stock pots heavily infected with *E. graminis* above them. The plants were left on a heavily dampened gravel bench and covered with sheets of newspaper for 24 hours due to intense sunlight creating very high temperature in the glasshouse. The plants were uncovered and remained on the bench until sampling.

Sampling, 72 hours after inoculation, was by detaching the fourth leaf from the stem base using a razor blade. The leaf was then cleaned using the method described previously and stained using 0.1% trypan blue. The basal 15cm of the leaf was cut into 5 cm sections and mounted on glass microscope slides adaxial side uppermost. A microscope was used to examine the sections and spores were counted and classified according to their stage of development. The intention was to carry out an analysis of variance on the data. As the data were percentages they deviated assumptions of normality required for this analysis. Therefore it was planned to arcsin transform these data before being analysed by analysis of variance.

Photo-micrographs were taken on a Leitz PMR microscope fitted with a Wold 48 photo system supplied by Leica using Boots 100 ASA colour print film. The standard operating procedure for the instrument was followed utilising automatic exposure control.

9.3 Results

Germination of spores was high for the untreated control and water control treatments at 85 and 89 percent respectively. The germination of spores for the potassium chloride and polyethylene glycol treatments was markedly lower at 38.1 and 28.9 percent respectively. The differences between the treatment were so clear that no analysis was carried out.

The percentage of the germinated spores producing haustoria was much lower for the potassium chloride treatment (12%) and polyethylene glycol treatment (13%) than for the control (98%) and water treatments (100%). On most of the replicates either none or all of the germinated spores produced haustoria. Therefore the data did not satisfy the requirement of normality assumed in analysis of variance. Accordingly statistical analysis was not conducted.

The *E. graminis* spores on the leaves treated with solutions of high osmotic potential exhibited a loss of size and became elongated in shape. This is illustrated for the potassium chloride treatment (Plate 17) and can be compared with an ungerminated spore on a control leaf (Plate 18).

The spores on the leaves receiving the control treatment or the water treatment developed normally and formed haustoria by the time the leaves were cleared and examined (Plate 21).

The osmotically active treatments usually resulted in no germination or the halt of germination at the production of very rudimentary germ tubes (Plate 19). On the limited occasions where spores did germinate they generally developed normally and formed an appressorium (Plate 20). However very few of the spores progressed beyond this stage. The few which did develop did not appear to do so normally and it appeared that the haustoria structures were not fully formed. However, the scarcity of the partially developed haustoria on the treated leaves meant that it was impossible to comment on the nature of the inhibition with great confidence. It appeared that the development of the haustoria was possibly inhibited by the application of potassium chloride or polyethylene glycol.

9.4 Discussion

It was very clear that treatment with a solution of high osmotic potential reduced the germination of *E. graminis* spores and prevented the remainder from completing the infection process. This was probably related to both surface effects and mechanisms inside the leaf. Visual observation suggested that the surface effect was the inhibition of spore germination and development, probably by causing an efflux of water from the spores resulting in a loss of turgor and in some cases plasmolysis. The loss of turgor pressure could be a factor in the reduced germ tube growth. A low turgor pressure would result in lower pressure

at the tip of the germ tube which would reduce the pressure needed for expansion and growth.

It appeared that those spores which did exhibit significant germ tube growth continued development normally with the formation of appressoria and infection pegs. This supported, as suggested previously, the possibility that the spores which do germinate and develop are located on areas of the leaf which were not covered with spray droplets. This scenario, coupled with an inability to form haustoria, suggests a mechanism in the leaf which might inhibit the development of haustoria. It was considered possible that the penetration of ions or molecules from the solutions might have increased the water potential of the leaf and hence created osmotic potentials which could have caused a water loss from the infection peg. This could have impeded the development of haustoria. Support for this concept comes from the few poorly developed haustoria-like structures developed on the spores on treated leaves. However this evidence must be considered inconclusive because of the poor resolution achieved in the photographs which renders interpretation difficult. The effect of applying solutions of high osmotic potential on leaf physiology and consequent effects on haustorial formation were considered to be a priority for further investigation.

Plate 17. An *Erysiphe graminis* f.sp *tritici* spore on a wheat leaf treated with a 64.14 bar solution of potassium chloride showing shrinkage and elongation.

Plate 18. An ungerminated *Erysiphe graminis* f.sp *tritici* spore on a wheat leaf receiving no treatment (control).

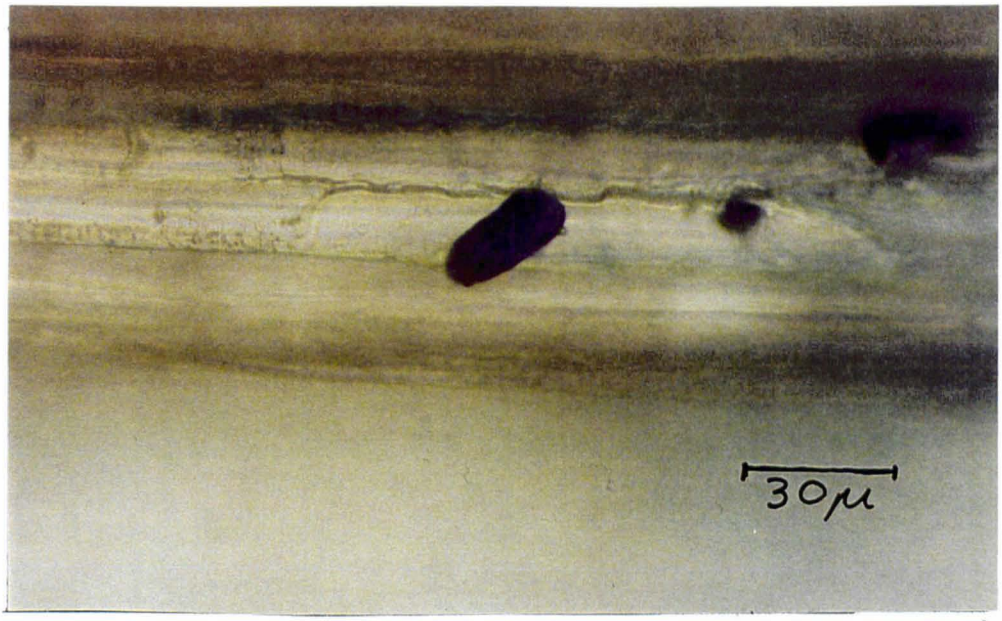


Plate 19. The development of rudimentary germ tubes by *Erysiphe graminis* f.sp *tritici* spore on a wheat leaf treated with a 64.14 bar solution of potassium chloride.

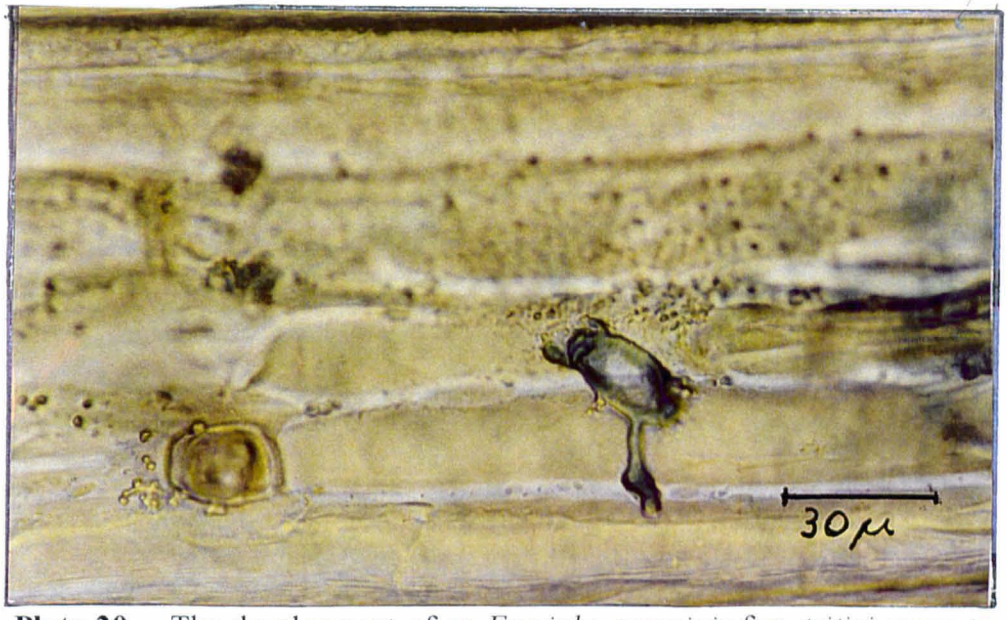


Plate 20. The development of an *Erysiphe graminis* f.sp *tritici* spore to appresorium formation on a leaf treated with a 64.14 bar solution of potassium chloride.

Plate 21. The development of an *Erysiphe graminis* f.sp *tritici* spore on a wheat leaf showing early haustorial formation 3 days after inoculation.

Plate 22. The development of an *Eryspihe graminis* f.sp *tritici* spore to early haustorial formation on a wheat leaf treated with a 64.14 bar solution of potassium chloride showing apparently abnormal development.

Chapter 10 - Investigations into the Uptake of Foliar Applied Potassium Chloride and Polyethylene Glycol by Wheat Leaves (Experiment UP1)

10.0 Introduction

The experiments relating the osmotic potential of foliar applied solutions (Chapter 8), the germination of *E. graminis* and leaf area affected by powdery mildew indicated that the disease control achieved with potassium chloride was not solely due to leaf surface effects. This assertion was supported by the observation that applying potassium chloride sprays to leaves one week before or after inoculation with *E. graminis* spores provided equivalent control. Spore germination would be complete before the application of a spray one week post inoculation (Smith, *pers.com.*). Equally it was suspected that a solution of potassium chloride applied one week before inoculation would have been almost completely taken up by the leaf within one week. If uptake was complete within one week potassium chloride could therefore have no surface effect on spore germination.

Although Aragues, Rogo and Grattin (1994) indicated that barley leaves would take up chloride ions and Chamel (1988) indicated that maize would take up potassium, no literature was found appertaining to the uptake of potassium chloride by wheat. Therefore an experiment was undertaken to investigate the uptake of potassium chloride and polyethylene glycol under controlled conditions to provide an indication of the speed and extent of uptake.

10.1 Objective

To investigate the uptake of potassium, chloride and polyethylene glycol by the leaves of wheat and test the following hypotheses.

1) The quantity of potassium present on a wheat leaf surface declines with time after application.

2) The quantity of chloride present on a wheat leaf surface declines with time after application.

3) The quantity of polyethylene glycol present on a wheat leaf surface declines with time after application.

4) The uptake characteristics of potassium, chloride and polyethylene glycol are similar.

10.2 Materials and methods

Wheat plants, cv. Apollo, were grown as described in general materials and methods with ambient air during October and November 1994. The plants were removed from the propagator at the commencement of the experiment and selected for uniformity. The unvernalised plants, which had five fully expanded leaves, were then randomly allocated to treatments using random numbers before being placed in controlled environment chambers for five days before the commencement of the experiment. The controlled environment cabinets were maintained at a constant 15 ^{o}C and 98% relative humidity with constant lighting from three fluorescent tubes.

The experiment was conducted in three controlled environment cabinets, each of which was considered a block. There were two factors which were solutions of 10% w/v potassium chloride or 53.6% w/v polyethylene glycol (average molecular weight 200) solution applied to the leaves. Treatments were considered to be sampling times.

Each cabinet or block contained sixty five plants. Five plants were designated untreated controls. Half (30) of the remaining plants were treated with

potassium chloride and half (30) with polyethylene glycol. The 64.14 bar solutions were applied as ten equally spaced four microlitre droplets to the upper side of the basal fifteen centimetres of the upper fully expanded leaf using a micro pipette and the plants placed in the cabinets in random arrangement. The leaves were held in place and horizontal by a length of florists wire attached to a cane. The wire was twisted around the cane at the height of the fifth leaf. It then pointed perpendicular to the cane. Two loops were twisted in the wire at approximately 8cm intervals. The leaf was threaded through the loops.

Samples were taken pre-treatment (untreated control), immediately after treatment and 3, 6, 12, 24 and 48 hours after treatment. At each time five replicates per block were removed and the upper leaf detached with a scalpel. Each leaf was then washed for five seconds in 20 ml of de-ionised water. The wash water was then frozen at -18 ^{o}C in boiling tubes until analysis. This proved to be a minor mistake as a few samples were lost when the glass cracked during freezing. However glass had to be used as plastic tubes may have contaminated the samples.

The samples from the potassium chloride treatments were analysed in two stages. A sub-sample was analysed for potassium concentration using a Gallenkamp flame photometer. Another sub-sample was prepared and analysed for chloride concentration using an EDT Instruments ion selective electrode with a silver chloride reference electrode. This was coupled to a Unicam 9450 pH meter set to record millivolts.

The samples from the polyethylene glycol treatments were analysed by gas chromatography using a Perkins Elmer 8500 chromatograph fitted with a 25m SGE BP20 polyethylene, bonded phase, polar capillary column with a 0.32mm internal

diameter and 0.25 micron film thickness. The chromatograph was run on an iso-thermal temperature programme for 50 minutes at 160°C with injector and detector temperatures of 260°C. A flame ionisation detector (FID) was used. The carrier gas was helium at a pressure of 8 PSIG. An external standard was used and the peak for polyethylene glycol with a molecular weight of two hundred appeared after approximately 4 minutes. Duplicate sub-samples were used for each analysis.

The results of all samples, except the pre-treatment control, were analysed by analysis of variance with polynomial contrasts.

10.3 Results

The decline in the quantity of potassium on the leaf surface was essentially linear (P=0.001) with a quadratic trend (P=0.001). It was clear from the results that the decline of potassium on the leaf surface was rapid over the first twelve hours after application and then slowed (Figure 24) (Appendix B7).

The decline in the quantity of chloride on the leaf surface followed a very similar pattern to that exhibited by potassium (Figure 25) (Appendix B8). The uptake was very rapid over the first three hours and then slowed. Uptake virtually ceased after twelve hours. This gave a very strong quadratic trend to the data (P=0.001). A cubic trend indicated by the analysis was due to a high value after 24r hours which was attributed to experimental error.

The decline in the quantity of polyethylene glycol was slower than that of the inorganic ions but still showed both linear (P=0.001)and quadratic trends (P=0.001) over the duration of the experiment (Figure 26) (Appendix B9).

Figure 24. The decline over time of potassium ion concentration washed from the leaves of winter wheat plants (cv. Apollo) in a standard volume of water following the application of potassium chloride. (bar = S.E.M.)

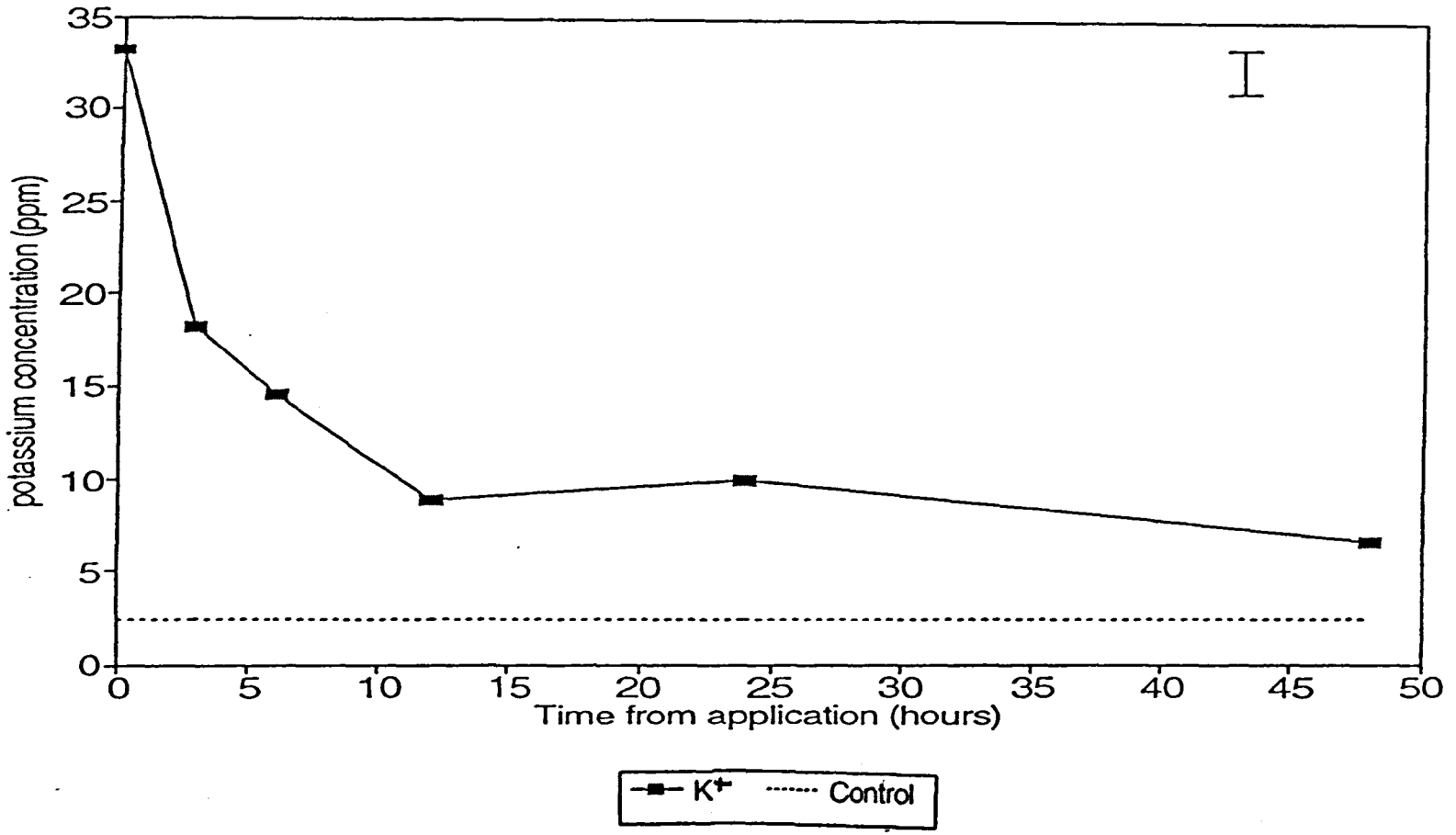


Figure 25. The decline over time of chloride ion concentration washed from the leaves of winter wheat plants (cv. Apollo) in a standard volume of water following the application of potassium chloride. (bar = S.E.M.)

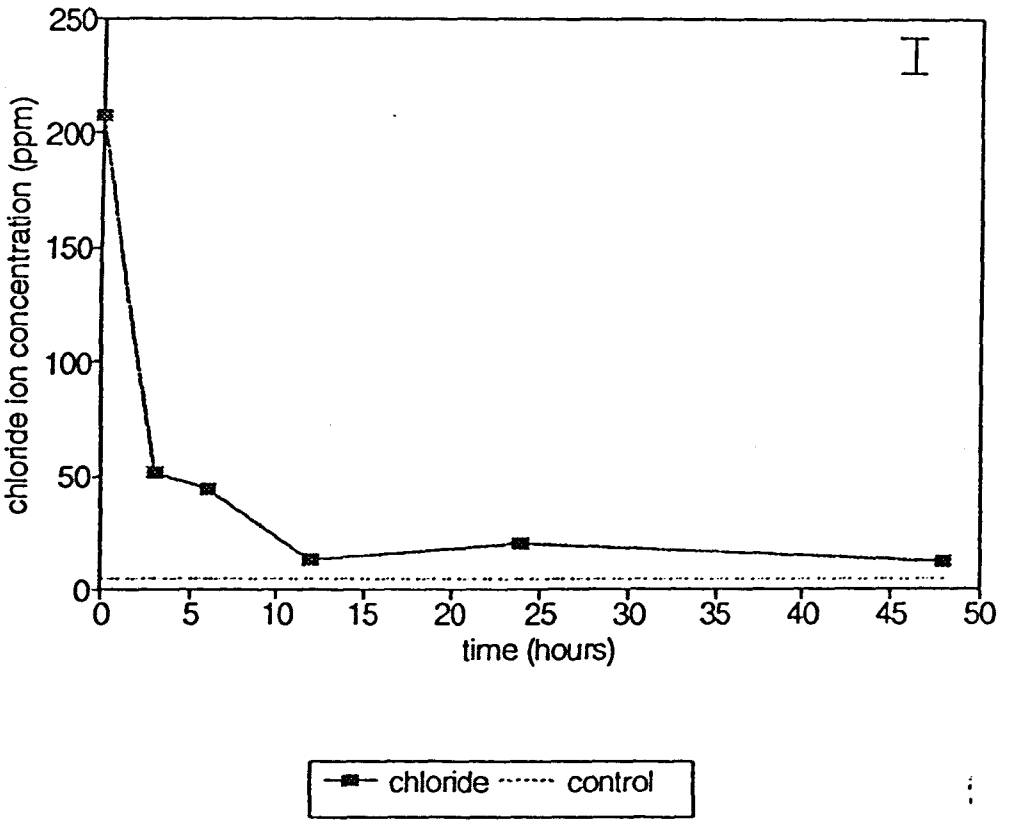
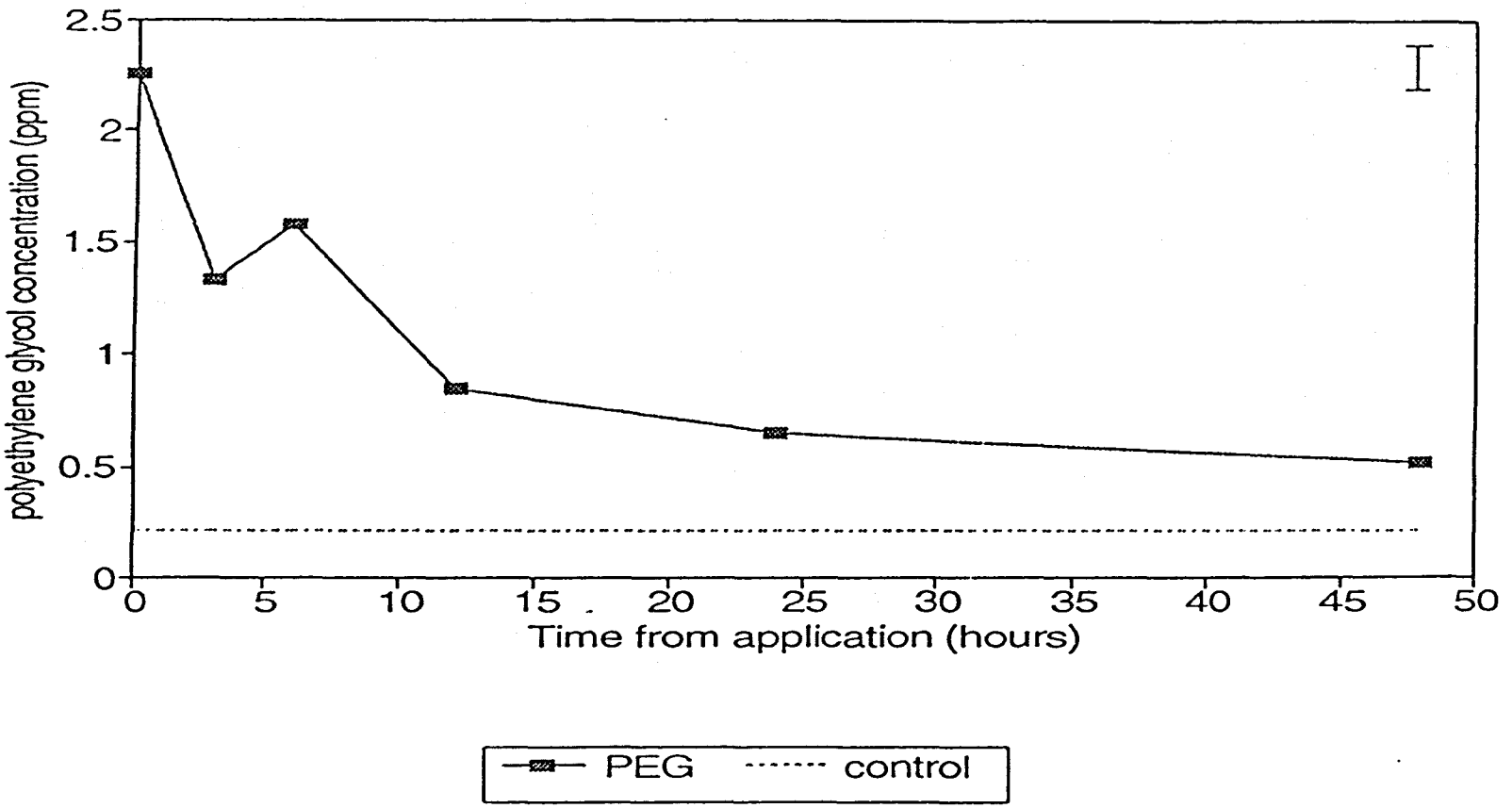


Figure 26. The decline over time of polyethylene glycol concentration on the leaves of winter wheat (cv. Apollo) washed in a standard volume of water following the application of polyethylene glycol solution. (bar = S.E.M.)



10.4 Discussion

Since the only reasonable explanation for the decline in the quantity of the compounds on the leaf surface was uptake by the plant the experiment indicated that potassium, chloride and polyethylene glycol are taken up by the leaves of winter wheat in a very short period of time. This rapid uptake suggested that the residence period of potassium chloride on the leaf surface is too short to account for the control of powdery mildew by plasmolytic activity against spores and hyphae when it is applied several days prior to inoculation. This suggested that a mechanism not involving surface activity may be involved in the control of powdery mildew by foliar applied potassium chloride. The rapid uptake of foliar applied potassium chloride suggested that this mechanism may occur inside the leaf through physiological changes in the host.

Over 10 to 20 hours the concentration of ions on the leaf surface declined five-fold. Since the activity of potassium chloride and polyethylene glycol solutions against powdery mildew is directly related to their osmotic potential it is likely that their activity will decline. Even if concentration was maintained by decreasing quantities of moisture on the leaf surface (unlikely at a relative humidity exceeding 95%) the quantity of solution and hence coverage of the leaf surface would decline as well, reducing effectiveness.

The fact that the residues from foliar application of potassium chloride and polyethylene glycol have fallen to a very low and relatively constant level after 48 hours (yet control can be achieved by applications 7 days before inoculation with *E. graminis*) suggests that the effect of these solutions on the establishment of powdery mildew is not totally dependent upon surface activity.

Chapter 11 - The Effect of Foliar Applied Potassium Chloride Solution On The Water Potential Of Wheat Leaves Over Time (Experiment LWP3).

11.0 Introduction

The application of foliar potassium chloride to wheat leaves prior to inoculation with spores resulted in the suppression of disease symptoms. If the indications of the previous experiment were generally applicable, the majority of the foliar potassium chloride would have been absorbed by the leaf and removed from the surface (Experiment UP1, Chapter 10). This suggested that inhibition of spore germination was not the only mechanism by which foliar applied salts could reduce the severity of powdery mildew infection.

It was suspected that the application of potassium chloride to the foliage caused changes in leaf water potential. The duration of the change in leaf water potential was considered an important factor in the mechanism by which the application of potassium chloride could reduce the severity of powdery mildew on wheat. An experiment was conducted to provide this information.

11.1 Objective

1) To ascertain any changes in leaf water potential resulting from the foliar application of potassium chloride.
2) To ascertain the duration of leaf water potential changes over time following the foliar application of potassium chloride.

11.2 Materials and Methods

Leaf water potential can vary widely due to changes in environmental conditions. Due to the unavailability of a controlled environment cabinet, a control treatment of distilled water was compared with a foliar spray of potassium chloride. Both were applied with a precision pot sprayer at 210 l/ha.

The plants used were in F84 module trays (Plantpak, Maldon, England) grown in the spore-free propagator. Each tray contained 84 unvernalised wheat plants (cv. Apollo) with three fully expanded leaves.

The two trays were cut in half; one half, allocated by tossing a coin, was sprayed with the equivalent of 210 l/ha 10% w/v potassium chloride solution as described in general materials and methods using a precision pot sprayer at 9.30am. The other half, designated the control, was sprayed with 210 l/ha de-ionised distilled water. The trays were then put back together on a glass house bench and maintained at soil moisture capacity. This was achieved by watering with a watering can fitted with a spout and taking care to avoid wetting the foliage.

Three solution treated and three control plants were selected using random number tables at each sampling time. These plants were destructively sampled by severing the upper fully expanded leaf just above the ligule with a scalpel. The leaf was placed in a pressure bomb (M. Cantwell, Reading) and the water potential was determined using pressurised nitrogen gas. The pressure was slowly raised until droplets of sap appeared on the cut end of the leaf. This was recorded.

The samples were taken at time zero, 3, 6, 12, 24, 72, 172 and 312 hours after treatment. The experiment was analysed by analysis of variance with

polynomial contrasts as a split plot design with two treatments (two main plots each with two sub-plots).

11.4 Results

The results from the samples taken over the first 24 hours of the experiment clearly showed the influence of diurnal changes on the leaf water potential of the wheat (Figure 27). There was a significant overall difference (P=0.01) (Appendix B10) between the two treatments with the leaf water potential being consistently higher in plants receiving foliar applications of potassium chloride than in the controls. These differences were apparent from six hours after the application of treatments and were still evident after 172 hours.

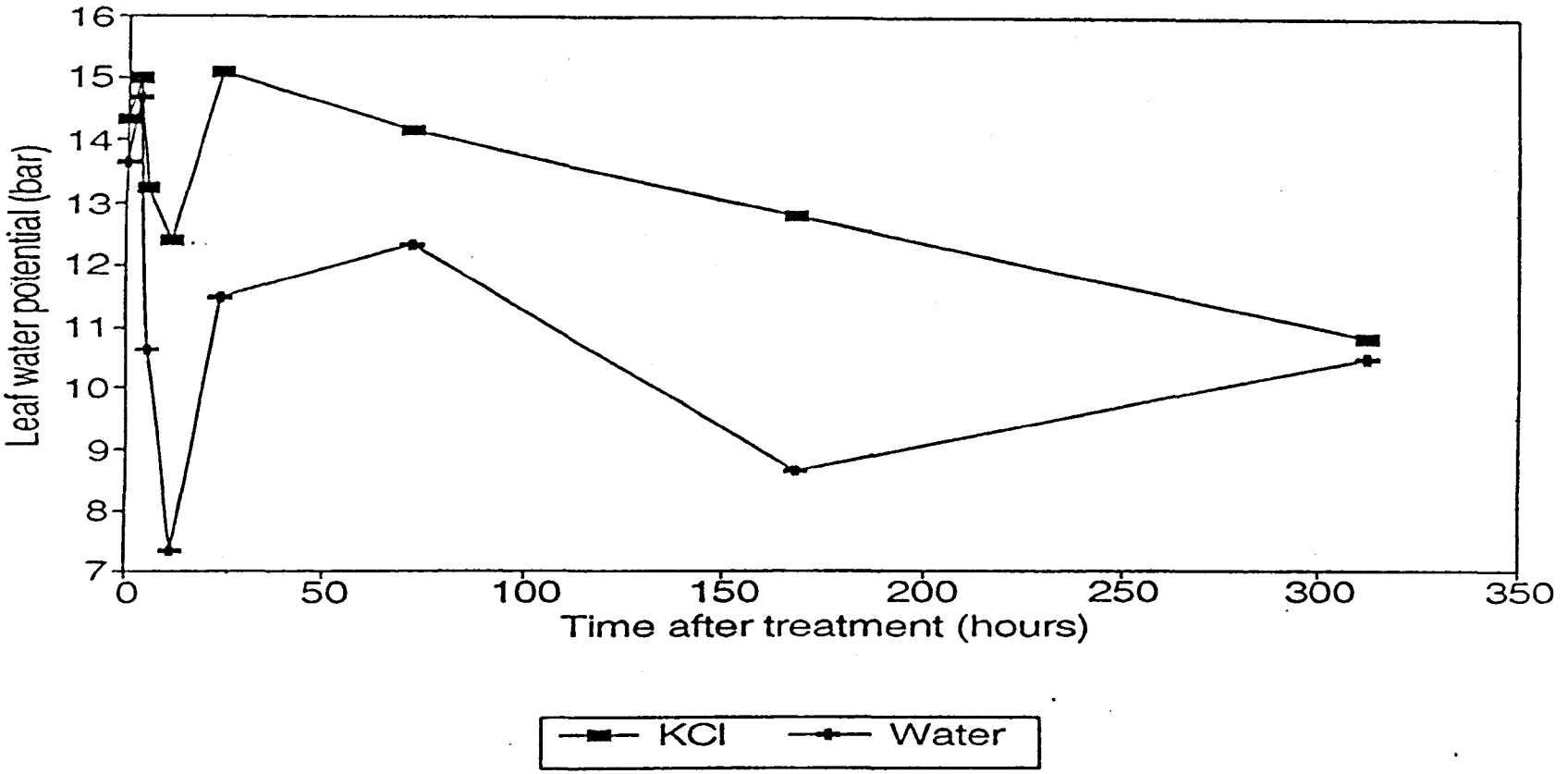
11.5 Discussion

It was clear that the application of potassium chloride solution and as a foliar spray to the foliage of wheat under typical glass house conditions could cause an increase in the leaf water potential compared to a control for a period in excess of a week. In the light of previous experiments (Chapter 5) duration of these effects was sustained enough to suggest that changes in leaf water potential may be connected to control of powdery mildew on wheat.

Polyethylene glycol was not included in this experiment due to a shortage of plant material. Due to the similarities in uptake profile with time and similar efficacy at reducing leaf area affected by powdery mildew, one might expect similar trends. However this experiment was considered to be peripheral to the main project. It confirmed that foliar application of potassium chloride could produce changes in leaf

water potential over a period of time, which was what was expected from the uptake experiments (Chapter 10). It was not considered necessary to repeat this experiment with polyethylene glycol.

Figure 27. Experiment LWP3.The effect over time of foliar applications of water and 10% w/v potassium chloride solutions at a rate equivalent to 210 l/ha on the water potential of the third leaf from the stem base of unvernalised winter wheat (cv. Apollo).



Chapter 12 - Investigations Into The Relationship Between The Osmotic Potential Of Foliar Applied Solutions, Leaf Water Potential And The Severity Of Powdery Mildew Disease On The Leaves Of Wheat (Experiment REG1).

12.0 Introduction

For the reasons described above it was thought that internal changes in leaf water potential might affect the establishment of *E. graminis* infections. The previous work had shown that potassium, chloride and polyethylene glycol were taken up by the leaf and could change the leaf water potential for a period of time. It was decided to evaluate the effect of applying osmotically active solutions on the leaf water potential and relate this to the leaf area affected. It was thought that the change of water potential would alter with the concentration or osmotic potential of the solutions applied. If this did occur then it was possible that this may, at least in part, explain the decline in the percentage leaf area of winter wheat leaves affected by powdery mildew which occurs as the osmotic potential of the solution applied is increased.

12.1 Objective

1) To examine the relationship between the osmotic potential of foliar applied solutions and the leaf water potential of wheat leaves.
2) To investigate the relationship between the osmotic potential of foliar applied solutions and the percentage area of wheat leaves affected by powdery mildew caused by *Erysiphe graminis*.

3) To examine the relationship between leaf water potential and leaf area affected by powdery mildew.

12.2 Materials and Methods

Unvernalised wheat plants (cv. Apollo) were grown in the manner described in general materials and methods. The plants were selected at the four leaf stage and allocated to treatments using random numbers.

The experiment had two factors: osmoticum and osmotic potential. The two osmotica were potassium chloride and polyethylene glycol. These were applied at five levels which were solution osmotic potentials of 12.83, 25.65, 38.48, 51.31 and 64.14 bar solutions (made up as detailed in Chapter 8, page 164). An untreated and a water sprayed control were also included in the experiment outside the factorial design. All treatments were applied at a volume equivalent to 215l/ha. Treatments were applied in the early afternoon and the plants laid out in 6x4 grid pattern blocks. Each treatment was applied to two plants per block. The blocks were arranged in a 2x5 grid on the glasshouse bench giving ten replicates for each assessment. The plants were inoculated using stock pots in the usual manner as soon as the leaves were visually free of moisture. All plants were kept watered to field capacity using a watering can with a spout to avoid wetting the foliage.

After twenty four hours the fourth leaf from the stem base was detached from one plant of each treatment per block and the leaf water potential was determined immediately.

Leaf water potential was determined using nitrogen gas in a pressure bomb (M. J. Cantwell, Reading)(Plate 23). The leaves were detached using a scalpel and placed in the pressure bomb as described in Chapter 10. The pressure was slowly elevated and a record taken of the pressure at which bubbles of sap were expressed from the cut end of the leaf.

The fourth leaves from the stem bases of the remaining plants were assessed for leaf area affected by powdery mildew, fourteen days after inoculation, by visual estimation using A.D.A.S assessment keys for reference (Anon, 1976).

Both sets of data were analysed using analysis of variance with polynomial contrasts. The relationship between leaf area affected and leaf water potential was examined using a linear regression model which accounted for block effects and differences in the response to osmoticum.

12.3 Results

There was a significant difference between the treatments applied with respect to leaf area affected by powdery mildew on the upper and lower leaf surfaces (P=0.001) (Appendices B11 and B12). The response of leaf area affected by powdery mildew to osmotic potential of the solution was a highly significant negative linear trend on both leaf surfaces (P=0.001) (Figure 28 and Figure 29).

There was a significant osmotica * osmotic potential interaction (upper surface P=0.01; lower surface P=0.001) indicating a differential response to solutions of given osmotic potential made with different osmotica. However, no clear pattern was apparenton the lower side of the leaf. On the upper side of the leaf there was a

significant linear * deviation interaction (P=0.05) indicating a differential response to osmotica which changed with osmotic potential. Examination of the graphed data (Figure 28) suggested that potassium chloride solutions of low osmotic potential gave better control of powdery mildew than polyethylene glycol of equivalent osmotic potential. At higher solution osmotic potentials this difference declined.

Leaf water potential responded to osmotic potential of the foliar applied solution with a highly significant linear trend (P=0.001) (Appendix B13). However the response curves of the osmoticum differed indicated by significant Osmotica * osmotic potential interaction with linear and quadratic components(P=0.05) (Figure 30).

There appeared to be a relationship between leaf water potential and percentage leaf area affected. However, because the foliar applied solutions had already been shown to alter the percentage leaf area affected by influencing spore germination it was not possible to examine this relationship directly. It was necessary to use multiple linear regression to compare these variables.

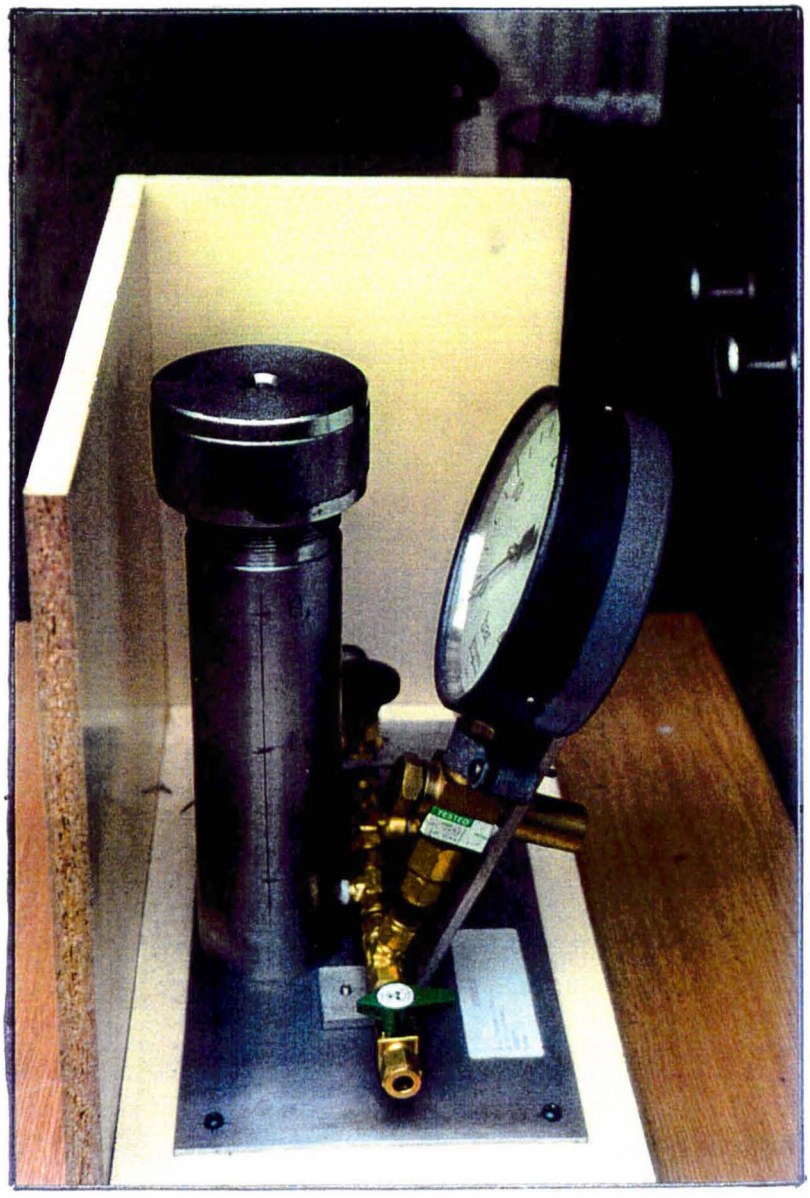


Plate 23. The pressure bomb.

Figure 28. Experiment REG1. The effect after fourteen days of solutions of different osmotic potential created with two osmotica, polyethylene glycol and potassium chloride, on the percentage area of the upper side of the fourth leaf from the stem base of winter wheat cv. Apollo. Affected by powdery mildew (bar=S.E.M.)

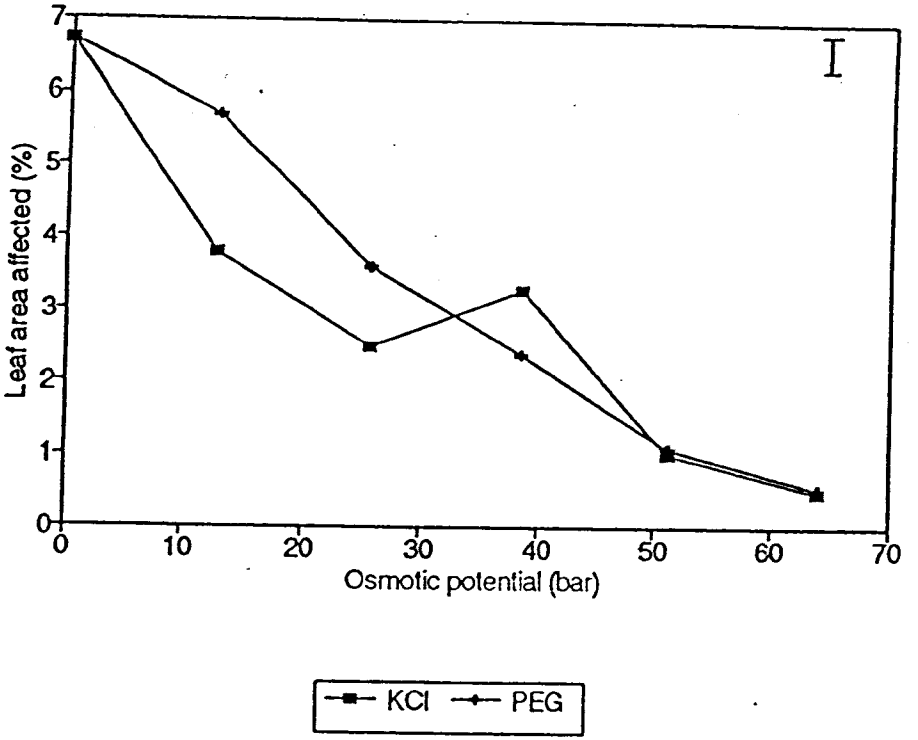


Figure 29. Experiment REG1. The effect after fourteen days of solutions of different osmotic potential created with two osmotica, polyethylene glycol and potassium chloride, on the percentage area of the lower side of the fourth leaf from the stem base of winter wheat cv. Apollo affected by powdery mildew (bar=S.E.M.)

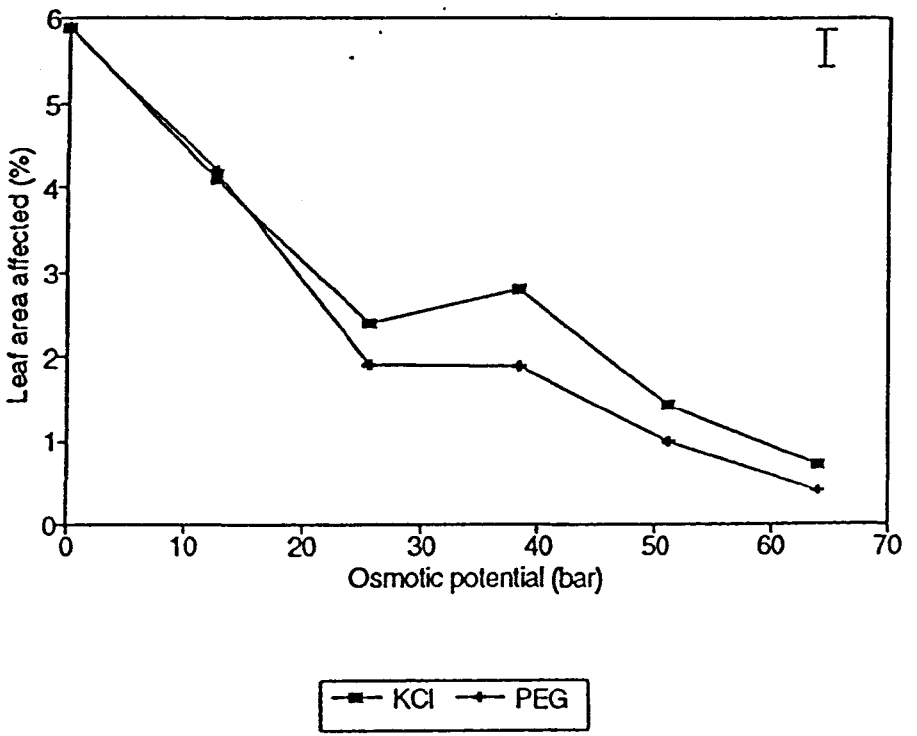
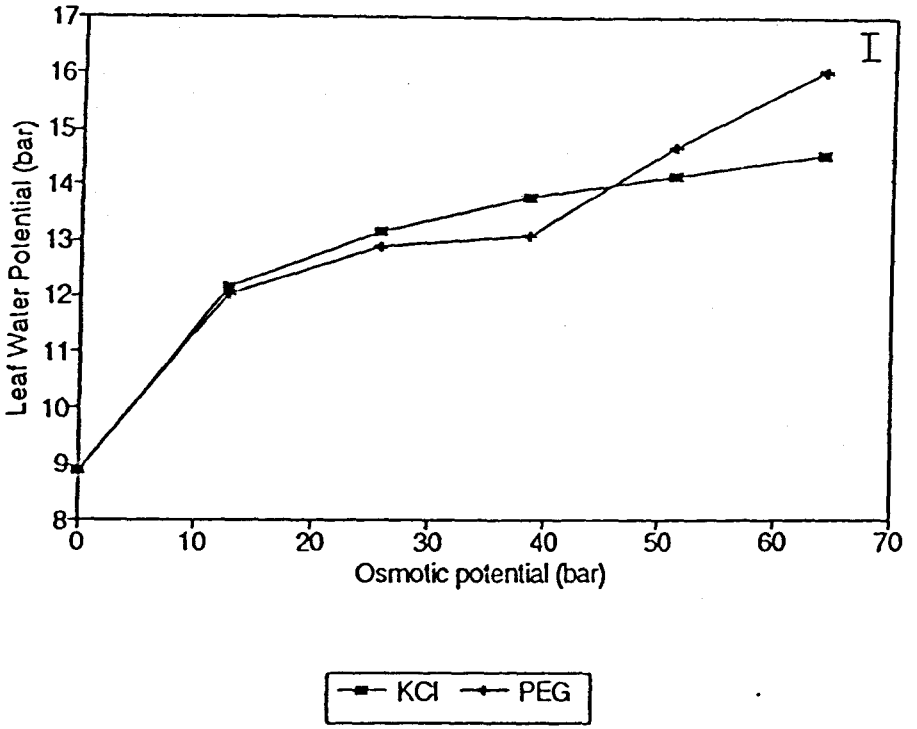


Figure 30. Experiment REG1. The effect after twenty four hours of solutions of different osmotic potential created with two osmotica, polyethylene glycol and potassium chloride, on the leaf water potential of the fourth leaf from the stem base of winter wheat cv. Apollo. (bar=S.E.M.)



12.4 Regression Modelling

A linear regression model was constructed to examine the relationship between leaf water potential and percentage leaf area affected. The objective was to construct a model which would account for as much of the variance in the data as possible and test the relationships between experimental factors and variables and the leaf area affected.

The initial terms accounted for in the model were block, because this was an overall source of variation affecting all data, and osmotica. The latter was included in the model due to the aforementioned differences in response to the variables leaf area affected and leaf water potential to increasing concentrations of polyethylene glycol and potassium chloride.

Since leaf water potential had a linear response to the osmotic potential of the solutions applied it was clear that any response to leaf water potential would be part of an overall response to osmotic potential. It was essential that the response to leaf water potential was examined before that of osmotic potential. Due to the interaction of osmotica with leaf water potential and percentage leaf area affected the next term fitted was a leaf water potential with osmotica interaction.

This left two possible terms which were osmotic potential and osmotic potential * osmotica interaction. Since leaf water potential had already been included in the model this term would account for effects on leaf area affected related to osmotic potential other than those attributable to induced changes in leaf water potential.

Analysis of variance to model terms goodness of fit to the data revealed that there was no significant relationship between leaf area affected, block and osmotica (Appendix B14).

There was a very significant fit of leaf area affected to a linear trend with regard to leaf water potential after accounting for block and osmotica effects (P=0.001). This indicated a very strong linear relationship between leaf water potential and leaf area affected. This relationship was according to the formula:-

$$LAA = LWP \times -0.141 + 3.25$$

where LAA = Percentage leaf area affected after 14 days

LWP = Leaf water potential after 24 hours

After fitting the other terms there was a probability (P=0.001) that the remaining variation in data fitted a linear response to osmotic potential. This indicated that although leaf water potential was a significant part of the osmotic potential effect on leaf area affected, a considerable part of the overall effect was due to mechanisms not directly relating to leaf water potential.

12.5 Discussion

It was confirmed again that the percentage leaf area of winter wheat, cv. Apollo, affected by powdery mildew declines in a linear manner with respect to increasing osmotic potential of the solutions applied to the leaf surface.

The leaf water potential of the wheat leaves increased linearly as the solution osmotic potential of the treatments increased. The slight differences in the polyethylene glycol and potassium chloride in this increase may be due to their different uptake characteristics.

The application of osmotically active solutions to the leaf surface increased leaf water potential and decreased leaf area affected. Modelling indicated that this relationship was linear but that the reductions in leaf area affected were not due solely to the induced changes in the leaf water potential and was partially dependent upon another mechanism, or mechanisms, related to the osmotic potential of the treatments. This was not entirely unexpected because previous experiments had suggested that spore germination declines in contact with solutions of high osmotic potential. It seemed likely therefore that the reductions in the leaf area of wheat affected by powdery mildew were due to two mechanisms: a probable initial effect upon the spores germinating on the leaf surface and a secondary mechanism which prevents spores which germinate establishing an infection and forming haustoria. The conclusion of this and previous experiments suggests that osmotica are taken up by the leaf and cause a change in water potential. This then creates conditions in which powdery mildew is unable to establish a parasitic relationship. It was therefore decided to examine this concept with a further experiment.

Chapter 13 - Investigations Into The Relationship Between The Osmotic Potential Of Foliar Applied Solutions, Spore Germination, Leaf Water Potential And The Severity Of Powdery Mildew Disease On The Leaves Of Wheat (Experiment REG2).

13.0 Introduction

The experiments described above revealed that leaf water potential increased if solutions of high osmotic potential were applied as a foliar spray. The higher the osmotic potential of solutions applied the greater the increase in leaf water potential. There was a very significant linear correlation with the visual estimations of percentage leaf area affected. However, it was considered essential to repeat the experiment. The occurrence of co-related effects such as the decline of spore germination as the osmotic potential of foliar sprays of fertilisers were increased, suggested that the experiment should be modified to improve the predictive model. As previous experiments had indicated that solutions with a high osmotic potential had very significant effects on the germination of spores on the leaf surface it was considered imperative that the experiment examining the relationship between leaf water potential and percentage leaf area affected was repeated with an allowance made for the side effects of the solutions on spore germination.

13.1 Objectives

1) To determine the response of leaf water potential to the application of solutions of differing osmotic potential.
2) To determine the response of spore germination to the application of solutions of differing osmotic potential.
3) To determine the relationship between the osmotic potential of solutions applied to wheat leaves, spore germination, leaf water potential and leaf area affected by powdery mildew.

13.2 Materials and Methods

The experiment had two factors which were foliar sprays of either potassium chloride or polyethylene glycol, each at five levels which were osmotic potentials of 12.83, 25.65, 38.48, 51.31, and 64.14 bar. Two treatments, an untreated control and a spray of distilled water were included in the experiment but outside the factorial design. Three responses were measured: leaf area affected by powdery mildew, leaf water potential and spore germination.

The plants used were four leaf unvernalised wheat plants (cv. Apollo). These were grown during February and March 1995 using the methods detailed in general materials and methods. The plants were pre-selected for even growth.

Plants were allocated to blocks and treatments using random numbers. There were nine blocks laid out as a 3x3 grid. Each block contained thirty six plants laid out on a 6x6 pattern. There were three plants for each treatment. One plant was allowed to go through to disease symptom development, the other two plants were destructively sampled for leaf water potential and spore germination determinations after twenty four and eighteen hours respectively.

Leaf water potential and spore germination were determined as described previously. Leaf area affected was assessed after 14 days by visual estimation aided by A.D.A.S. charts (Anon, 1976).

The individual data sets were evaluated using analysis of variance with polynomial contrasts. The spore germination data was arcsin transformed before analysis. The combined data sets were analysed using a multiple regression model to compare the effects and separate the influences.

13.3 Results

Spore germination declined with increasing solution osmotic potential in a quadratic manner (P=0.05) (Appendix B15) (Figure 31). There was a difference between the slopes of the quadratic curves reflecting a different response to the two osmotica with potassium chloride giving slightly lower reductions than polyethylene glycol. However the responses appeared linear above 25 bar in line with previous results.

Leaf water potential had a positive linear relationship with solution osmotic potential (P=0.001) (Appendix B16) although the slope of the potassium chloride response was steeper than that of the polyethylene glycol (P=0.001) (Figure 32).

There was a negative linear relationship between the percentage area of the upper (P=0.001) (Appendix B17) and the lower (P=0.01) (Appendix B18) sides of the fourth leaf from the stem base affected by powdery mildew (Figures 33 and 34).

Because of the effect of solution osmotic potential measured on three variables it was necessary to compare the relationships simultaneously in one analysis where the fit of leaf area affected to the other variables could be considered after the removal of sources of variation not of immediate concern.

13.4 Regression Model

A multiple linear regression model was constructed using the Genstat programming package (Version 5). Block was the initial term specified in the model as it was the most generalised source of variation and affected all treatments equally. Due to the interactions between osmoticum and responses to increasing solution osmotic potential with respect to leaf area affected, leaf water potential and percentage spore germination this was added to the model.

Both percentage spore germination and leaf water potential had linear responses to the increase in solution osmotic potential. It was therefore considered

that both of these responses would be included in a global response of leaf area affected to solution osmotic potential. Therefore, they were fitted to the model in advance of the solution osmotic potential.

The percentage spore germination was fitted before leaf water potential on biological grounds. The solution makes contact with the leaf surface where the spores are located, and would affect their germination before penetrating the leaf to cause changes in the leaf water potential.

The final term to be fitted was solution osmotic potential and solution osmotic potential by osmotica. Thus the final order of model terms was:-

Block + Osmotica + Spore Germination + Spore Germination.Osmotica + Leaf Water Potential + Leaf Water Potential.Osmoticum + Solution Osmotic Potential + Solution Osmotic Potential.Osmotica

The analysis of variance testing for fit to the model indicated that there was not a significant fit with regard to blocks and osmotica.

There was a good fit to the linear model ($P = 0.05$) (Appendix B19) for spore germination and percentage leaf area affected after accounting for the effect of variation incorporated in blocks and osmotica effects. There was no significant difference between osmotica with regard to the fit of spore germination to leaf area affected.

The fit of leaf area affected to leaf water potential was not significant at the 5% level but was at the 10% level. Although normally this would not be considered conclusive in this case it may be considered that the response of leaf area affected to leaf water potential was tending towards a linear relationship because of the evidence provided by previous experiments.

Figure 31. Experiment REG2. The effect of appplying solutions of different osmotic potential, created with potassium chloride or polyethylene glycol to wheat cv. Apollo as a foliar spray on the germination of E. graminis spores on the fourth leaf from the stem base eighteen hours after treatment.

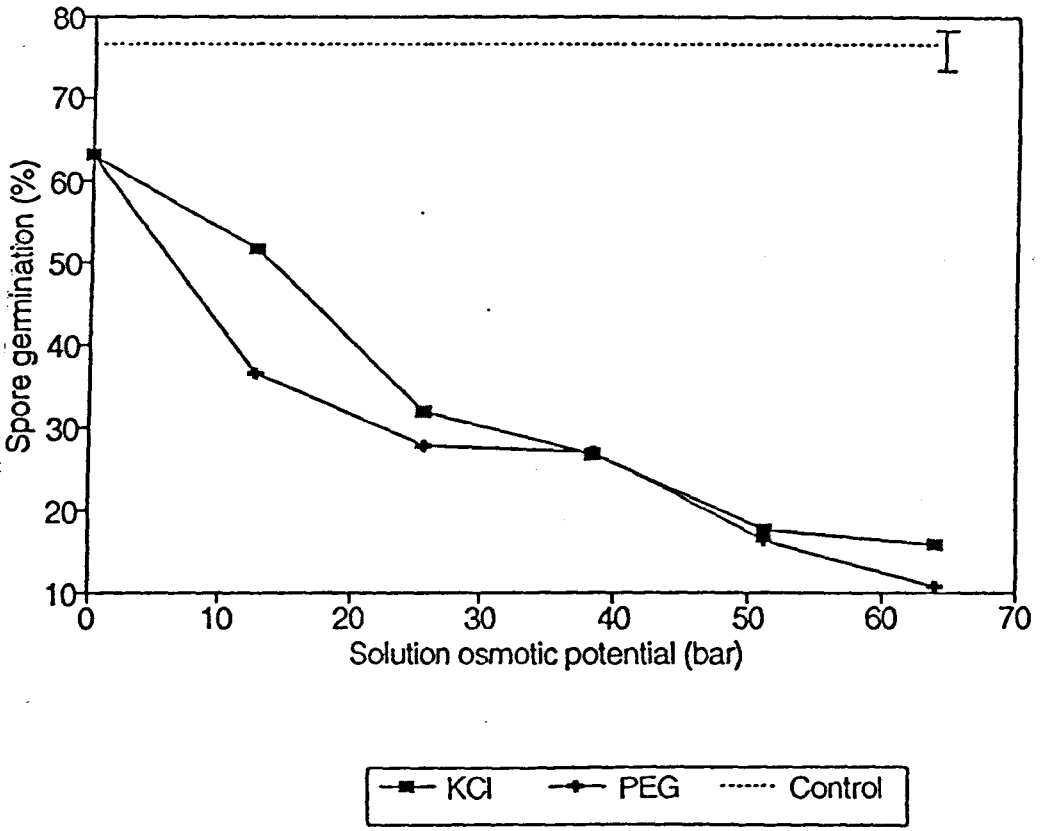


Figure 32. Experiment REG2. The effect of applying solutions of different osmotic potential, created with potassium chloride or polyethylene glycol to wheat cv. Apollo as a foliar spray on the leaf water potential of the fourth leaf from the stem base twenty four hours after treatment. (bar = S.E.M.)

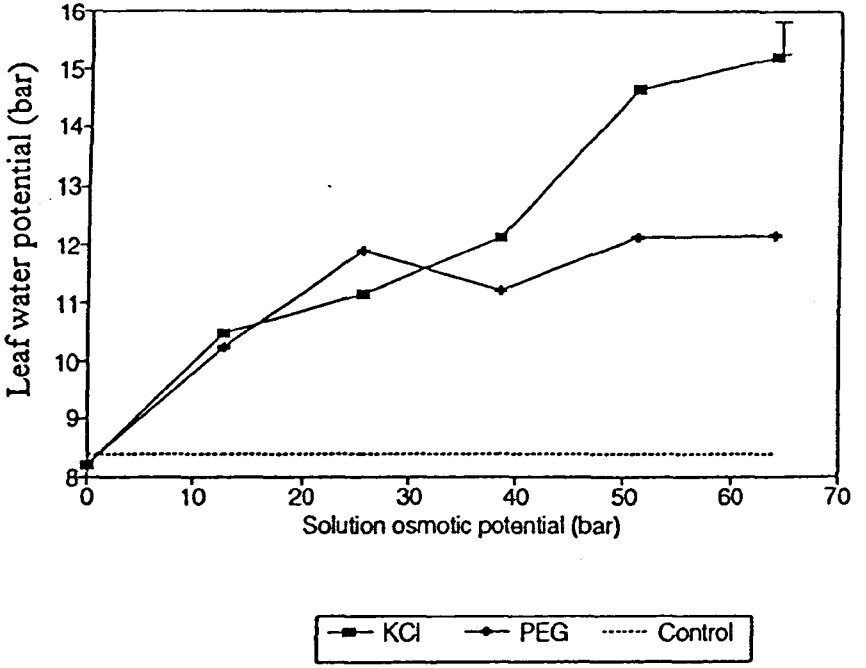


Figure 33. Experiment REG2. The effect of applying solutions of different osmotic potential, created with potassium chloride or polyethylene glycol to wheat cv. Apollo as a foliar spray on the percentage area of the upper side of the fourth leaf from the stem base affected by powdery mildew. (bar = S.E.M.)

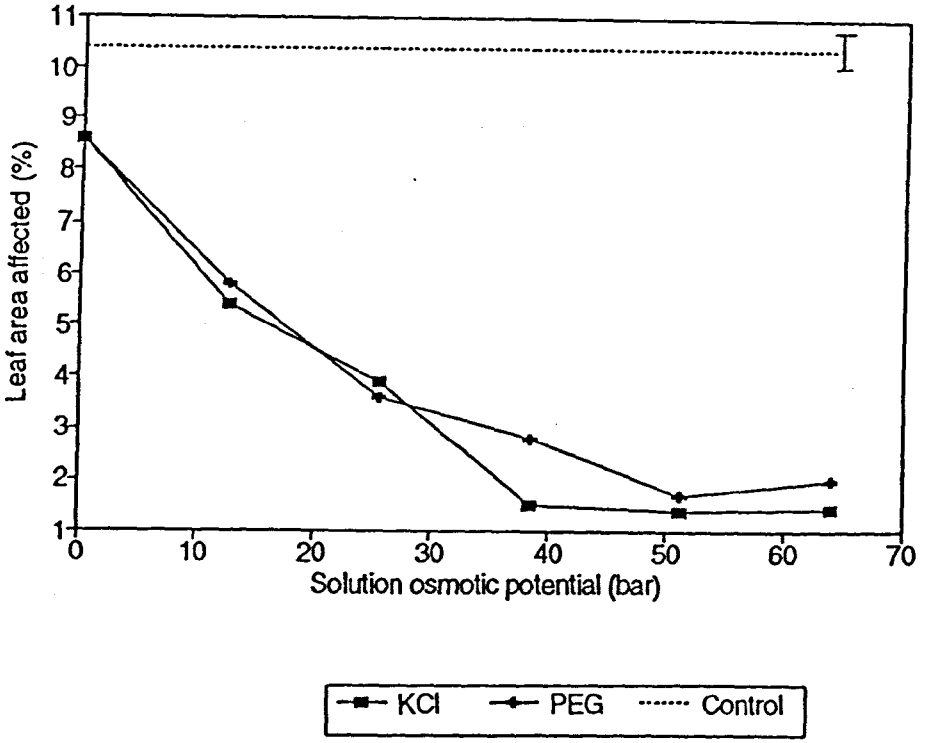
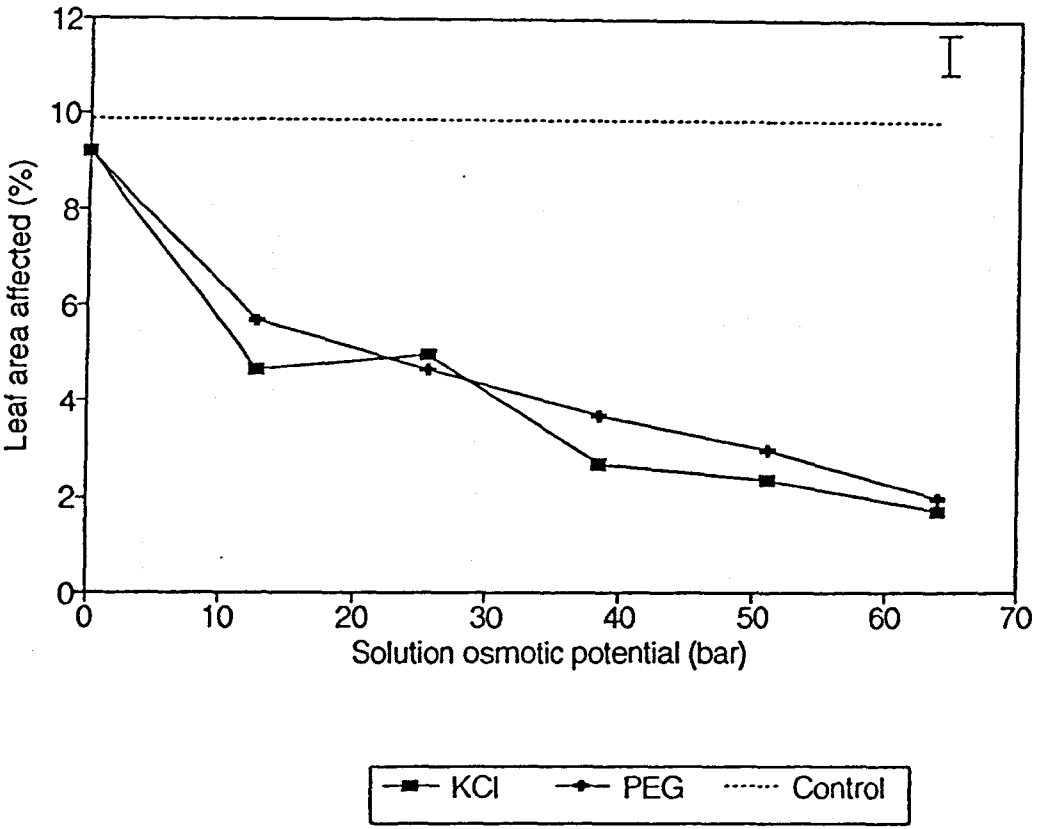


Figure 34. Experiment REG2. The effect of applying solutions of different osmotic potential, created with potassium chloride or polyethylene glycol to wheat cv. Apollo as a foliar spray on the percentage area of the lower side of the fourth leaf from the stem base affected by powdery mildew. (bar = S.E.M.)



13.5 Discussion

The analysis of the data again indicated strong linear relationships between solution osmotic potential, leaf water potential, percentage spore germination and leaf area affected. The increase in leaf water potential and decrease in percentage spore germination with increasing solution osmotic potential suggested that they could be effects of applying the fertilisers which might reduce leaf area affected.

If even a small percentage of the total spores on a leaf were to germinate this should potentially give rise to some quite high levels of leaf area affected by powdery mildew colonies.

Examination of the data illustrated in Figure 31 indicates that complete inhibition of spore germination was not achieved. However, almost complete control of powdery mildew, manifested as an absence of visual symptoms, was achieved by the treatments with the highest osmotic potentials.

The applications of solutions of high osmotic potential resulted in a rise of leaf water potential and for the reasons explained previously it was reasonable to expect this to result in a reduction in pathogen establishment. Previous experiments had indicated a negative linear relationship between leaf water potential and leaf area affected.

The multiple regression model indicated that spore germination had a very significant effect on the leaf area affected which responded with a linear decline. It was tentatively suggested in this case that the remainder of the control was due to the increase in leaf water potential. The fit of leaf water potential to the linear model was only significant at the 10% level rather than the level accepted as significant under normal circumstances. However previous work had suggested a relationship between leaf water potential and percentage leaf area affected and the correlation coefficient of -0.412 did indicate some degree of correlation between spore germination and leaf water potential which may have resulted in some proportion of the leaf water

potential effect being accounted for by the spore germination term in the model. Therefore it can be concluded that this experiment did not unequivocally indicate that percentage leaf area affected by powdery mildew had a linear relationship with leaf water potential. However the analysis did indicate a possible tendency towards a linear relationship. However this cannot be asserted with confidence.

Therefore it would appear that the activity of solutions of potassium chloride on powdery mildew is due to a physico-chemical activity related to the solution osmotic potential. This activity is principally due to the solution on the leaf surface creating osmotic potentials which draw water from the spores causing an inhibition of spore germination. In the light of previous experiments and very tentative evidence from this experiment the solutions enter the leaf causing an increase in leaf water potential and this might possibly be responsible for a secondary reduction in the leaf area affected by powdery mildew.

The absence of significant source effects in the model, and the consideration that polyethylene glycol is widely regarded as biologically inert, indicated that potassium chloride is not a metabolic poison but acts only by physico-chemical mechanisms. Any differences between responses to the application of potassium chloride and polyethylene glycol are probably explained by their different rates of uptake into the leaf as described previously, which might in turn influence the speed with which leaf water potential changed in response to treatment.

Chapter 14 - General Discussion

14.0 Objectives

The general objectives of this project were to identify a foliar fertiliser which could significantly reduce foliar disease in cereals permitting a reduction in fungicide usage and identify the mechanism behind the observed effects on disease.

Constraints imposed on the investigation were that the fertiliser must supply a nutrient normally required by the crop and be applied by a conventional hydraulic sprayer at a volume of less than two hundred and fifty litres per hectare. These objectives were to be achieved by literature review, field and laboratory experiments. Literature review revealed urea and potassium chloride as the most likely compounds for the control of foliar disease on cereals. Investigation identified potassium chloride to be the more effective compound. It was considered that the first objective was achieved. The second objective was partially achieved during this investigation.

14.1 Urea

Urea solutions were evaluated by field trial and proved partially successful On wheat foliar application of urea was found to cause a small decrease in powdery mildew symptoms. Unfortunately the foliar application of urea to barley increased the severity of powdery mildew (*Erysiphe graminis*) and brown rust (*Puccinia recondita). S.tritici* was also increased on wheat. These increases in disease severity led to the conclusion that urea was unsuitable as an agent for reducing fungicide usage on cereals. The results differed from those of Gooding *et al.* (1989) who found that mildew, along with other diseases, was consistently reduced on wheat.

These differences may have been explained by the mechanisms causing the phenomena observed.

Penny *et al.* (1978) suggested that 'scorch' or phytotoxic damage caused by the application of urea resulted in a disruption of the host pathogen relationship. This hypothesis was not supported but even if the membranes were not affected some form of physiological disturbance must arise to cause 'scorch'. Work by Spalding (*pers com*) suggested that urea was rapidly absorbed by the leaf and decomposed to ammonia which was a highly phytotoxic compound causing cellular dysfunction. In some cases with biotrophic pathogens the disruption may be such that the maintenance of a relationship with the host was rendered impossible and overall disease severity was reduced. The cellular dysfunction caused by urea may depress the normal defensive mechanisms used by the leaf allowing pathogens greater opportunity to establish.

The different response of barley and wheat to urea application with respect to powdery mildew possibly reflected differences in tolerance of enhanced ammonia levels in the leaf. It appeared possible that barley was less tolerant to the physiological stress placed on it by an application of urea than wheat and consequently this led to it being more susceptible to powdery mildew infection than similarly treated wheat.

Applying foliar urea to the leaf also supplies nitrogen which can have a purely nutritional effect on disease severity. The field experiments conducted were controlled in that foliar treatments were evaluated in comparison to equivalent soil applications.

The field experiments described in this work indicated that foliar application of urea solution increased the percentage leaf area affected by *S. tritici*. This contrasted with the findings of Gooding *et al.* (1989) who found that foliar applications of urea reduced the percentage leaf area affected by *S. tritici*. However, these workers applied foliar urea as a supplement. They did not have any treatments where an equivalent application of urea was made to the soil. Therefore it would appear that increasing the supply of nitrogen to a wheat plant by any route can decrease the percentage leaf area affected by S. tritici. However, these are at least partially offset by some deleterious factors associated with foliar application.

14.2 Potassium Chloride

The objectives of the field experiments with potassium chloride were to challenge a range of field epidemics and observe any effects that the foliar fertiliser had upon disease levels.

It was clearly evident that potassium chloride reduced foliar disease severity in winter wheat over two years. Both powdery mildew and *S. tritici* were significantly reduced. However, control of disease by a spray applied at flag leaf fully emerged was evident in only one year. This was probably because the level of disease in the second year trial was low after stem extension and differences were not apparent between any treatments except variety. The reduction in flag leaf disease achieved in experiment WKCL1 was in agreement with Kettlewell *et al.* (1990). However the control of both powdery mildew and *S. tritici* by the application of potassium chloride sprays at stem extension was a new finding and showed that foliar

applied potassium chloride could reduce disease incidence early in the growing season.

Yield responses were not achieved in either year. This was partly in agreement with Kettlewell *et al.* (1990) who reported inconsistent yield responses to foliar application of potassium chloride with yield responses occurring only on very potassium deficient sites and not associated with significant disease reduction. Other workers reported inconsistent yield responses by cereals to potassium fertiliser (Anon 1994a). One of the problems encountered in the selection of sites was the relatively high potassium contents of the local soils which severely limited site choice. The requirements of cereals for potassium are satisfied at very low soil concentrations, with very small differences between deficient and non deficient sites, and less fertile sites would have been preferred as these would have been more likely to show differences in response to yield and disease symptoms to fertiliser treatments.

In view of the significant effects both *S. tritici* and powdery mildew can have on cereal yield (Carver and Griffiths, 1981; Thomson and Gaunt, 1986), the reductions in leaf area affected by *S. tritici* and powdery mildew during early stem extension, achieved by foliar application of potassium chloride, might have been expected to give significant yield responses even on soil containing adequate available potassium.

Taking into account the minor contribution made by the lower leaves to crop yield (Anon, 1981) it was thought that the crops in the experiments compensated for the potential yield loss caused by early infection. This was supported by the findings of Thomson and Gaunt (1986).

Another possible reason for the lack of yield response may have been the yield potential of the experimental site. The only field sites with low soil potassium concentrations available were very light sandy loam sites with limited water retention capacity. In these experiments water supply may have been the first yield limiting factor. Therefore any yield response to disease control would not have been exhibited. In the second experiment there were no differences in yield between treatments except by variety. This supported the suggestion of site limitation. The yield responses in the first experiment were confounded by very unusual weather conditions including water logging post anthesis. This experiment was grown after cereals and although not assessed quantitatively, examination of the culms after harvest revealed high levels of stem base discolouration attributed to a stem base disease complex. It was believed that the yield responses to fungicides were due to the control of stem base diseases rather than the control of foliar pathogens.

The application of potassium chloride to the foliage and to the soil in both field and glasshouse experiments revealed that the foliar spray resulted in disease reductions but soil applications did not. In some instances a water spray reduced leaf area affected by powdery mildew in the glasshouse but not in the field. Even in these instances the reduction was never as great as that achieved with very weak potassium chloride solutions. Therefore it was considered that the control of powdery mildew observed in all experiments was due to some factor intrinsic to the solution rather than the water or salt components individually.

In glasshouse experiments the decline of leaf area affected by powdery mildew was usually linear when solution concentration was increased from zero to approximately 10% w/v. This further suggested that the intrinsic factor in the

solution responsible for decreasing the severity of powdery mildew was dependent upon the number of ions present in the solution. This suggested that the control was by one of three routes; one or both of the ions in the solution was directly fungicidal, the solution had a physico-chemical effect on the pathogen on the leaf surface or the ions in the solution were absorbed in the leaf and altered its osmotic potential thereby inhibiting pathogen development. The latter two mechanisms were considered the most likely and easiest to test.

Tests conducted *in vitro* using mildew spores exposed to a range of osmotic potentials created with polyethylene glycol and potassium chloride revealed that increasing the solution osmotic potential increased the percentage of cells with visible plasmolysis. These responses were linear suggesting a physico-chemical response. However germination was also reduced in a linear manner suggesting a link between this and plasmolysis. There was no difference between the two osmotica This indicated that solution osmotic potential was the critical factor and the constituent ions of potassium chloride were not directly toxic to the pathogen spores. This was later confirmed *in vivo* where a strong negative linear correlation was found between the osmotic potential of the foliar applied potassium chloride solution and the percentage leaf area affected by powdery mildew. These studies indicated that solution osmotic potential could inhibit spore germination *in vitro*. However, the plasmolysis of spores *in vitro* in solutions of high osmotic potential was not unexpected and the conditions *in vitro* are considered different to those encountered *in vivo* on the leaf surface. Therefore the results of the two investigations were not directly comparable in a quantitative manner although the general trends appeared similar.

The initial experiments conducted *in vivo* examining spore germination and leaf area affected indicated two things. The reduction in leaf area affected showed a linear response to solution osmotic potential and was not markedly different with regard to the source of osmotic potential. Secondly the reduction in leaf area affected appeared, at least in part, to be due to the solutions reducing the percentage germination of the spores. This indicated clearly that the effect of foliar applied potassium chloride on powdery mildew was by physico-chemical means, not as a metabolic process affecting either the host or the pathogen. However, the examination of spores on treated leaves revealed that many spores survived treatment with even the most concentrated solutions used. This contrasted sharply with the almost complete eradication of visible leaf symptoms when high concentrations of potassium chloride were applied. The number of spores surviving were more than sufficient to establish colonies which would be expected to result in much higher percentage leaf areas affected by powdery mildew than those actually observed. This suggested that other mechanisms were involved in the inhibition of *E. graminis*. The surviving spores almost invariably produced infection pegs. However, examination of treated leaves several days after inoculation and treatment showed that very few spores produced haustoria on leaves treated with 64.14 bar solutions of potassium chloride or polyethylene glycol.

This suggested that the inhibition was the result of at least two mechanisms. The first appeared to be by the inhibition of spore germination on the leaf surface by causing the loss of water and hence turgor in the *E. graminis* spores. This caused a reduction in spore germination. The spores which did germinate generally developed normally up to penetration of the leaf. This suggested that these spores survived

because they were not in contact with the foliar applied solutions. Since the spray was applied at a medium droplet size complete coverage of the leaf was highly unlikely (Mathews, 1979).

The remaining mechanism was at this stage unknown but the normal development of spores on the leaf surface, coupled with an inability to form normal haustoria, suggested a mechanism in the leaf epidermal cells which prevented the establishment of a parasitic relationship with the host plant.

This latter mechanism may also explain why control can be achieved by an application seven days after treatment. In this case infection processes would already be complete with haustoria formed and hyphal proliferation occurring prior to the emergence of visible symptoms. This has been shown to occur under the prevailing conditions (Chapter 9). It was obvious that hyphae on the leaf surface might be plasmolysed as suggested by Weeds *et al.*, (1993). However to prevent the resurgence of symptoms the focus of the infection, the haustoria, would have to be destroyed. The hyphae are isolated from the haustoria by a septum in the haustorial neck (Bracker, 1968).

The most probable cause of haustorial dysfunction is a change in the osmotic potential of the cell sap following the uptake of salts by the leaf. The most likely mechanism by which osmotic changes in the cell could destroy the haustorium concern the structure of the haustorial complex. The haustorial complex consists of the haustorium which projects into the cell wall of the host cell causing an invagination of the host plasmalemma but separated from direct contact, except possibly at the tips of the haustorial lobes, by a matrix of pectic and hemicellulosic substances (Gil and Gay, 1977). The extra-haustorial membrane is isolated from the

rest of the plasmalemma by the secretion of a collar of material resembling a casparian strip (Bracker, 1968). It has been proposed that the extra-haustorial membrane is semi-permeable but passes no enzymes for active transport (Spencer-Philips and Gay, 1981). These workers suggested that a high ATPase activity on the haustorial membrane indicated enzyme-mediated active transport of substrate from the extra-haustorial matrix creating a diffusion gradient which would be replenished by influx from the host cytoplasm. An alternative suggestion is that the translocation could be by active transport (Manners, 1979).

In either case, the influx of inorganic ions into the cytoplasm of epidermal cells following the application of a foliar fertiliser would create an increase in their osmotic potential. This in turn would result in a movement of water from the extra-haustorial matrix into the host cell. This would cause the concentration of soluble substances, including sucrose, in the matrix to rise. This would reduce any inflow of sucrose by diffusion and might provide a negative feedback signal to reduce the active transport of metabolites. Either possibility would leave the pathogen nutritionally deficient. Furthermore, the increased concentration of solutes in the extra-haustorial matrix would result in an increase in its osmotic potential and a net outflow of water from the fungus. The overall effect of this may be to debilitate the fungus preventing the maintenance of an infection.

It was demonstrated that potassium chloride and polyethylene glycol were taken up by the leaf causing the osmotic potential of the vascular sap to be altered.

Furthermore, experiments indicated that the leaf water potential of wheat leaves increased with the applications of increasingly concentrated foliar sprays of solutions to the plant. These differences were maintained for over two weeks. These

findings suggested that the mechanism proposed above might occur. Multiple linear regression indicated that although the inhibition of spore germination was the primary mechanism by which foliar applied potassium chloride reduced disease severity there was an indication that induced changes in leaf water potential of the leaf might be related to the leaf area affected. This latter finding was not completely conclusive. It is possible that induced changes in leaf water potential become more important when the foliar fertiliser is applied at a time further from inoculation than that used in these experiments.

Therefore it appeared that surface activity was probably only part of the mechanism by which foliar applied potassium chloride reduced disease. The multiple linear regression experiments showed that effects on spore germination and leaf water potential accounted for virtually all of the decline in disease severity resulting from the foliar application of potassium chloride solution. Potassium chloride and polyethylene glycol were therefore shown not to be metabolic poisons of *Erysiphe graminis*.

14.3 Benefits and Potential of Potassium Chloride as a Material for Reducing Fungicide Usage

In the field, foliar applied potassium chloride reduced the leaf area affected by foliar disease compared with a soil applied control treatment. The actual severity of disease and the reduction achieved was not significantly different from those achieved with a conventional full rate fungicide programme. This initially suggested that the use of foliar potassium chloride could reduce the need for fungicides.

However in both years yield responses to the application of foliar potassium chloride did not occur. This was attributed to two factors. The application of potassium chloride to the foliage of wheat plants at the concentrations used in the field experiments was above the optimum concentration used in glasshouse experiments. Although it is common for conventional fungicides to provide equivalent control when applied at lower concentrations in the glasshouse than the field (Parry, *pers. com.*) this was not necessarily true with fertilisers. Above the optimum concentration used in the glasshouse experiments it was suspected that phytotoxicity was occurring. If this was the case the plants in the field experiment may have had any yield benefit from the control of disease negated by the stress caused by the fertiliser. The alternative reason was site limitation as already discussed.

The evidence collected indicated that the foliar application of potassium chloride could provide control of *E. graminis* and *S. tritici* similar to that provided by commercially available fungicides. However, further work is needed to determine whether or not foliar applied potassium chloride can produce similar yield responses in favourable conditions. The foliar applications of potassium chloride may sometimes replace conventional fertiliser applications, meaning that no extra fertiliser is used, although commercial hydroponic grade totally soluble potassium chloride costs £300 per tonne (Kemira Horticulture, *pers. com.*) compared to conventional fertiliser grade at £105 (Nix, 1993) approximately trebling the cost. However if the fertiliser replaces a conventional fungicide application the foliar fertiliser can be applied at no extra cost and the cost of spreading granular fertiliser is saved. The greatest potential saving is reduced expenditure on conventional fungicides.

However, this is difficult to quantify on a general basis because the prices of conventional fungicides vary annually and there is a large range of alternative products.

As the effect of potassium chloride sprays against stem base diseases, especially eyespot, is unknown it is probable that potassium chloride sprays would be used in an integrated program with conventional fungicides, so reducing any potential cost saving.

It may be argued that the application of potassium chloride at flag leaf emerged is too late to influence yield. However, many cereal growing soils in the United Kingdom are index two or higher and need potassium containing fertiliser only to maintain soil reserves for the next crop. Assuming that straw is not removed this could be achieved by a foliar spray of potassium chloride.

A further advantage of the foliar fertiliser as an agent for disease control concerns operator safety. Potassium chloride solution is non volatile and of low mammalian toxicity other than by ingestion. These features make it particularly safe to use compared with conventional fungicides.

The research is at an early stage but it is possible to suggest that foliar applied potassium chloride may have a role in crop protection to reduce the overall level of inputs and reduce costs. It was also possible that it might be acceptable for use in environmentally motivated production schemes where the use of conventional synthetic fungicides are restricted.

14.4 Future Work

This relationship between the application of potassium chloride as a foliar spray and the leaf area of wheat affected by powdery mildew needs considerably more investigation.

The work would involve further investigation of the mechanisms by which the inhibition of *E. graminis* is achieved and more applied research is needed to improve the efficiency of the fertiliser as a disease control agent.

Regarding the mode of action studies, the effect of potassium chloride solutions on the leaf surface are well established. The effect of induced changes in the leaf water potential are less well investigated. The logical progression of this project would involve repeating the final experiment described in Chapter 13 with certain changes. Applying the foliar treatments seven days before or after inoculation would ensure that the solutions were not on the leaf surface around spore germination. This would reduce the relative importance of effects of spore germination and enable the effect of leaf water potential on disease to be examined with reduced co-effect from the solution.

With regard to enhancing the effectiveness of foliar applied potassium chloride as a disease control agent several areas need further attention.

The studies on the mode of action revealed the inhibition of spore germination on the leaf surface to be a major component of the total effect. The incomplete control of spore germination on the leaf surface was attributed to incomplete coverage of the leaf surface by potassium chloride or polyethylene glycol solutions. This indicates that the effect could possibly be enhanced by the use of surfactants to lower the surface tension of the spray droplets enabling the solution to

spread more evenly over the leaf surface. An experiment examining the effect of a range of surfactants on the inhibition of spore germination and disease severity could be conducted. In view of the expected enhancement of action the experiment should involve the use of a range of fertiliser concentrations.

If the further experiments indicate that altering leaf water potential is critical in achieving control of established infections or providing protective activity the role of penetrants should be examined. The use of commercially available penetrants such as urea would increase speed and total uptake of potassium chloride and result in greater and more rapid activity. A further benefit of more rapid uptake would be a lower risk of rain washing the solution off the leaf in the field.

The final stage of the investigation regarding powdery mildew would depend on the testing of the most suitable formulations in field experiments to relate the field and glasshouse results. Ideally this would be conducted at locations where the yield potential of the site would not limit yield responses.

Foliar applied potassium chloride has been shown to reduce the symptoms of other cereals diseases. In this investigation control of *Septoria tritici* was shown to be possible. The physico-chemical nature of the mode of action against powdery mildew suggests that the solution could have broad spectrum activity against a range of cereal pathogens. To fully utilise the potential of potassium chloride as a disease control agent in integrated crop production these activities against other species ought to be investigated.

REFERENCES

Ahmad, S.A., Owera, S.A.P., Farrar, J.F. and Whitbread, R. (1982). The distribution of five major nutrients in barley plants infected with brown rust. *Physiological Plant Pathology.* **21**,335-346.

Anon. (1976). Manual of plant growth stages and disease assessment keys. Ministry of Agricuture, Fisheries and Food. Pinner. Middlesex. U.K.

Anon. (1981). *The use of fungicide and insecticides on cereals.* Booklet 2257. Ministry of Agricuture, Fisheries and Food. Pinner. Middlesex. U.K.

Anon. (1986). *The Analysis of Agricultural Materials. A Manual of the Analytical Methods Used by the Agricultural Development and Advisory Service.* 3rd edition. pp 191-193. Ministry of Agricuture, Fisheries and Food. Pinner. Middlesex. U.K.

Anon. (1994a). *Fertiliser recomendations for agricultural and horticultural crops (RB209)* Ministry of Agricuture, Fisheries and Food. Pinner. Middlesex. U.K.

Anon. (1994b). *Cereal varieties hand book,* NIAB recommended lists of cereals 1994. National Institute of Agricultural Botany. Cambridge UK.

Aragues, R., Rogo, A. and Grattin, S.R. (1994). Foliar uptake of sodium and chloride in barley, sprinkler irrigated with saline water: effect of pre irrigation with fresh water. *European Journal of Agronomy*,**1**,9-18

Bainbridge, A. (1974). Effect of nitrogen nutrition of the host on barley powdery mildew. *Plant Pathology.* **23**,160-161.

Barraclough, P.B. and Leigh, RA. (1993). Grass yield in relation to potassium supply and the concentration of cations in tissue water. *Journal of Agricultural Science, Cambridge.* **121**,157-168.

Bartlet, M.S. (1949) Biometrics from *Statistical Methods.* Snedecor, G.W. and Cochran, W.G., Iowa State University Press, Ames, Iowa.

Bracker, C.E. (1968). Ultrastructure of the haustorial apparatus of *Erysiphe graminis* and its relationship to the epidermal cell of barley. *Phytopathology*,**58**,12-30.

Burchill, R.T. and Cook, R.T.A. (1971). The interaction of urea and microorganisms in suppressing the development of perithecia of *Venturia inequalis*(Cke.)Wint., in *Ecology of Leaf Surface Microorganisms,* eds. T.F. Preece and C.H. Dickinson. pp 471-83 Academinc Press. London.

Butler, E.J. and Jones, S.G. (1949). *Plant Pathology,* Macmillan, London.

Caldicot, J.J.B. and Nutall, A.M. (1979). A method for the assessment of lodging in cereal crops. *Journal of the National Institute of Agricultural Botany*,**15**,88-91.

Carver, T.L.W. and Griffiths, E. (1981). Relationship between powdery mildew infection, green leaf area and grain yiedls of barley. *Annals of Applied Biology.* **99**,255-266.

Chamel, A. (1988). Foliar uptake of chemicals studied with whole plants and isolated cuticles, pp **27-50** in *Plant Growth and Leaf applied Chemicals* Ed. P.M. Neumann. CPC. New York

Christensen, N.W., Jackson,T.L. and Powelson, R.L. (1982). Suppression of take-all root rot and stripe rust diseases with chloride fertilisers. 111-116. in: *Proceedings 9th International Plant and Nutr. Coll.,Warwick Univ., England.* Commonwealth Agric. Burreaux. pp11-116.

Cook, J.W., Kettlewell, P.S. and Parry, D.W. (1993). Control of *Erysiphe graminis* and *Septoria tritici* on wheat with foliar applied potassium chloride. *Journal of the Science of Food and Agriculture.* **63,** 126.

Cooke, B.M. and Jones, D.G. (1970). A field inoculation method for *Septoria tritici* and *Septoria nodorum*. Plant Pathology. 19, 72-74

Corner, E.J.H. (1935). Observations on resistance to powdery mildews. *New Phytologist*, **34,** 180-200.

Daamen, R.A., Winjnands, F.G. and Vliet, G. van der (1989). Epidemics of disease and pests at different levels of agrochemical input. *Journal Phytopathology* **125**, 305-319.

Daly, J.M. (1949). Influence of nitrogen source on the development of stem rust of wheat. *Phytopathology* **39**, 386-394.

Darwinkel, A. (1975). Aspects of assimilation and accumulation of nitrate in some cultivated plants. *Agricultural Research reports (Waganingen)* **843,** 1-64.

Das, N.R. and Sen, S. (1975). Incidence of brown rust in transplanted wheat as affected by NPK fertilisers and crop rotation. *Indian Journal of Agricultural Science.* **41,** 872-875.

Eibner, R. (1985). Foliar fertilisation - important for productivity of modern plant cultivation. in *Foliar Fertilisation,* ed. A. Alexander. pp 3-16. B.A.S.F. Germany.

Fournet, J. (1969). Propeties et role du cirrhe du *Septoria nodorum* Berk. *Annales Phytopathologie.* **1,** 87-94.

Gaunt, R.E., Lim, L.G. and Thomson, W.J. (1982). The identification of disease constraints to cereal yield: crop/food loss appraisal report. *FAO Plant Protection Bulletin,* **1,**3-8.

Gheorgies, C. (1974). Contribution to the knowledge of the life history of *Septoria tritici* Rob. et Desm. I pycnidiospore germination. *Annale Institutulne de Cerectari Protectia Plantalore.***10,** 63-70.

Gil, F. and Gay, J.L. (1977) Ultrastructural and physiological properties of the host interfacial components of haustoria of *Erysiphe pisi in vivo* and *in vitro. Physiological Plant Pathology.***10(1)** 1-12.

Glynne, M.D. (1958). Effect of potash on powdery mildew in wheat. *Plant Pathology* 5, 15-16.

Gooding, M.J., Kettlewell, P.S. and Davies W.P. (1989). Disease suppression by late season urea sprays on winter wheat and interaction with fungicide. *Journal of Fertiliser Issues.* **5** (1), 19-23.

Grybauskus, A.P. and Sammons, D.J. (1988) Effect of chloride fertilisers on the development of powdery mildew of wheat. *Plant Disease.* **72**, 605-607.

Hashim, L.O. and Russell, G.E. (1982). Possible control of brown (leaf) rust on winter wheat by applying chlorides to the soil. *Cereal Rusts Bull.* **10**, 29-33.

Hewitt, E.J. (1975) Assimilatory nitrate reduction in plants. *Annual Review of Plant Physiology.* **26,** 73-100.

Heydecker, W. and Gibbins, B.M. (1978). The 'priming' of [vegetable] seeds [in the inert osmoticum polyethylene glycol to improve the synchronisation of germination]. *Acta Horticulturae,* **83**, 213-223.

Huber, D.M. (1978). Disturbed mineral nutrition. in: *Plant Pathology - An Advanced Treatise,* Vol. III, eds. J.G. Horsfall and E.B. Cowling, Academic Press, New York, pp 163-181

Huber, D.M. (1980). Role of nutrients in defence, in *Plant Pathology - An Advanced Treatise*, Vol. V, eds. J.G. Horsfall and E.B. Cowling, Academic Press, New York, pp 381-406.

Huber, D.M. and Arny,D.L. (1985). Interactions of potassium with plant disease, in *Potassium in Agriculture*, ed. R.D. Munson, American Society Agronomy. Madison WI. pp 467-488.

Huber, D.M. and Watson, R.D. (1974) Nitrogen form and plant disease. *Annual Review of Plant Pathology* 139.

Jenkins, J.E.E. and Morgan, W. (1969). The effect of *Septoria* diseases on the yield of winter wheat. *Plant Pathology*,**18**,152-156.

Jenkyn, J.F. (1977). Nitrogen and leaf diseases of spring barley. *Fertiliser Use and Plant Health, Proceedings of the 12th Colloquium of the International Potash Institute*. Izmir, Turkey, pp119-28.

Jenkyn, J.F. and Bainbridge, A. (1978). Biology and pathology of cereal powdery mildews, in *The Powdery Mildews*, ed. D.M. Spencer, Academic Press, London. pp 283-321.

Jenkyn, J.F. and Finney, M.E. (1981) Fungicides, fertilisers and sowing date. In *Strategies for the Control of Cereal Disease* eds. J.F. Jenkyn and R.T. Plumb, Blackwell Scientific Publications, Oxford.

Jenkyn, J. F., Finney, M.E. and Dyke, G.V. (1983). Effects of nitrogen fertiliser and tridemorph on mildew, growth and yield of spring barley. *Journal of Agricultural Science Cambridge.* **101,** 517-546.

Jenkyn, J.F. and Griffths, E. (1978). Relationships between the severity of leaf blotch (*Rhynchsporium secalis*) and the water soluble contents of barley plants. *Annals of Appied Biology.* **90**, 35-44.

Jenkyn, J.F. and Moffatt J.R. (1975) The effect of ehirimol seed dressing on yield of spring barley grown with different amounts of nitrogen fertiliser. *Plant Pathology* **24,** 47-57.

Jones, L. (1990). The effect of foliar applied urea on powdery mildew on barley. Post graduate diploma report. Harper Adams Agricultural College.

Jordan, V.W.L. and Tarr, H. (1977). Epidemiology of splash-dispersed cereal diseases. *Report of Long Ashton Research Station for 1977.* pp110-111.

Kapusta, E.C. (1968). Potassium fertiliser technology, in *The Role of Potassium in Agriculture,* eds. Y.J. Kilmer, S.E. Younts and N.C. Brady, American Society of Agronomy, Madison, U.S.A. pp 23-52.

Kettlewell, P.S., Bayley, G.L. and Domleo, R. (1990). Evaluation of late season foliar application of potassium chloride for disease control in winter wheat. *Journal of Fertiliser Issues.* 7 (1), 17-23.

Kettlewell, P.S., Blouin, G.L. and Boulby, G.L. (1992) Evaluation of potassium chloride solution against leaf diseases in barley. *Tests of agrochemicals and cultivars.* **13**, 18-20.

Kettlewell, P.S. and Juggins, S.A. (1992) Can foliar application of nitrogen fertiliser to winter wheat reduce nitrate leaching. *Aspects of Applied Biology* **30**, 103-108.

Last, F.T. (1953) Some effects of temperature and nitrogen supply on wheat powdery mildew. *Annals of Applied Biology* **40**, 312-322.

Last, F.T. (1954) The effect of time of application of nitrogenous fertiliser on powdery mildew of winter wheat. *Annals of Applied Biology.* **41(3)**, 381-392.

Last, F.T. (1962a) Effect of nutrition on the incidence of barley powdery mildew. *Plant Pathology.* **1**, 133-5.

Last, F.T. (1962b) Analysis of the effects of *Erysiphe graminis* DC. on the growth of barley. *Annals of Botany* **26**, 279-89.

Liebhardt, W.C. (1968) Effect of potassium on carbohydrate metabolism and translocation. in *The Role of Potassium in Agriculture*, eds. V.J. Kilmer, S.E. Younts and N.C. Brady, American Society of Agronomy, Madison, pp147-164

Lupton, F.G.H. and Sutherland, J. (1973). The influence of powdery mildew (*Erysiphe graminis*) infection on the development of four spring wheats. *Annals of Applied Biology.* **74 (1)**, 35-39.

Manner, J.M. and Gay J.L. (1978). Uptake of 14C photosynthates from *Pisum sativum* by haustoria of *Erysiphe pisi. Physiological plant pathology.* **12**, 199-209.

Manners, J.G. and Housain, S.M.M. (1963). Effects of temperature and humidity on conidial germination in *Erysiphe graminis. Transactions of the British Mycological Society.* 46(2), 225-234.

Matthews, G.A. (1979) Pesticide application methods. Longman, New York.

Mead, R. and Curnow, R. N. (1983). *Statistical Methods in Agriculture and Experimental Biology.* Chapman and Hall, London.

Murray, A.J.S. and Ayres, P.G. (1986). Infection with powdery mildew can enhance the accumulation of proline and glycinebetaine by salt stressed barley seedlings. *Physiological Plant Pathology.* **29,** 271-277.

Nix, J. (1993). *Farm Management Pocketbook.* Wye College, University of London.

Paulech, C. (1969). Einfluss des Getreidemehltauplzes *Erysiphe graminis* DC. auf die Trockensubstanzmenge und auf das Wachstum der vegetativen Pflanzenorgane *Biologica, Bratislava.* **24,** 709-19. (Abstract in *Review of Plant Pathology.* **49**, 1619.

Parker, S.R., Shaw, M.W. and Royle, D.J. (1992) The reliability of visual assessments of disease severity. Disease management in relation to changing agricultural practice. *Proceedings of Disease Management In Relation To Changing Agricultural Practice. Belfast*, pp 78-85.

Pate, J.S. (1973). Uptake, assimilation and transport of nitrogen compounds by plants (*Lupinus albus*) *Soil Biology and Biochemistry.* **5 (1),** 109-119.

Peltonen, J., Kittila, S., Peltonen-Sainio, P. and Karjalainen, R.(1991) Use of foliar-applied urea to inhibit the development of *Septoria nodorum* in Spring wheat. *Crop Protection.* **10,** 260-264.

Penny, A., Widdowson, F.V and Jenkyn, J.F. (1978). Experiments with solid and liquid N fertilisers and fungicides on winter wheat at Saxmundham in Suffolk 1976-9 *Journal of Agricultural Science Cambridge.* **100,** 163-173.

Powelson, R.L., Jackson, T.L. and Christensen, N.W. (1984). Enhanced suppression of take-all root rot of wheat with chloride fertilisers, in *Control of Soilborne Diseases : Proceedings 5th International Plant Pathology Congress.*

Quarmby, S.C. (1991). Manufacture of nitrogenous fluids, in *Fluid Fertiliser Science and Technology,* ed. D.A. Palgrave, Marcel Decker, New York.

Reuveni, R. and Rotem, J. (1973). Epidemics of *Leveillula taurica* on tomatoes and peppers as affected by the conditions of humidity. *Phytopathology Zeitshrift.* **2.** 25-33.

Ridgeman, W.J. (1975). *Experimentation in Biology*. Blackie, Glasgow.

Russell, G.E. (1978). Some effects of applied sodium and potassium chloride on yellow rust in winter wheat. *Annals of Applied Biology*. **90,** 163-168.

Sadler, R. and Scott, K.J. (1974). Nitrogen assimilation and metabolism in barley leaves infected with the powdery mildew fungus. *Physiological Plant Pathology*. **4.** 235-248.

Singh S. (1961). Effect of mineral nutrition on the incidence of net-blotch of barley. *Indian Journal Agricultural Science*. **33**, 1-7.

Spencer-Phillips, P.T.N. and Gay, J.L. (1981). Domains of ATPase in plasma membranes and transport through infected plant cells. *New Phytologist*. **89**,393-400.

Temiz, K. (1977). Interaction of fertilisers with Septoria leaf blotch of wheat. *Fertiliser Use and Plant Health, Proceedings of the 12th Colloquium of the International Potash Institute*, Izmir, Turkey, pp119-28.

Thomson, W.J. and Gaunt, R.E. (1986). The effect of speckled leaf blotch on apical development and yield in winter wheat in New Zealand. *Annals of Botany*. **58**,39-48.

Walters, D.R., and Ayres, PG. (1983). Changes in nitrogen utilisation and enzyme activities associated with CO_2 exchanges in healthy leaves of powdery mildew-infected barley. *Physiological Plant Pathology*. **23,** 447-459.

Weeds, P.L., Long, P.G., Ganeshanaddam, S., Hopcroft, D.H. and Bennett, R.J. (1993). Sodium bicarbonate and additives for the control of rose and curcubit powdery mildew. *6th International Congress of Plant Pathology, Montreal Canada.*

Williams, J.R. and Jones, D.G. (1972). Epidemiology of *Septoria tritici* and *S. nodorum*. VI. Effect of time of the initial infection on disease development and grain yield in spring wheats. *Transactions of the British Mycological Society.* **59**, 273-283.

Zaracovitis, C. (1966). Shrivelling and collapse of conidia of powdery mildew fungi. *Ann. Inst Phytopath. Benaki, N.S.* **7**, 219-226.

Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974). A decimal code of the growth stages of cereals. *Weed Research* **14**, 415-421.

APPENDIX A1.

Details of husbandry appertaining to all plots in Experiment BU1.

4.10.91 Drilled (depth 3.5cm) Seed rate 400 seeds/m^2

Cultivar Pastoral Dressing S.P.D.

11.11.91 GS21 Sprayed Panther herbicide 2.0 l/ha

13.3.92 GS30 Top dressing Prilled Urea 86.9kg/ha = 40kgN/ha

17.3.92 GS30 Sprayed Ceridor herbicide

Cycocel 5 C growth regulator

APPENDIX A2.

Details of husbandry appertaining to all plots in Experiment WU1.

4.10.91 Drilled (depth 3.5cm) Seed rate 400 seeds/m^2

Cultivar Apollo Dressing S.P.D.

(Hereward)

11.11.91 GS21 Sprayed Panther herbicide 2.0 l/ha

13.3.92 GS30 Topdressing Prilled Urea 86.9kg/ha = 40kgN/ha

17.3.92 GS30 Sprayed Ceridor herbicide

Cycocel 5 C growth regulator

17.4.92 GS32 Top dressing Prilled Urea 130.35kg/ha = 60kgN/ha

APPENDIX A3.

Details of husbandry appertaining to all plots in Experiment WKCL 1.

Location. Tank field, Burnhill, Green Farm, Patshull, Shropshire.

The following treatments were applied to all plots.

29.9.91 Drilled (depth 3.5cm) Seed rate 140 kg/ha

Cultivar Mercia Dressing S.P.D.

29.11.91 GS20 Sprayed Panther (IPU + diflufenican) herbicide

Sprayed Isotop (HBN) herbicide

Sprayed Cypermethrin insecticide

3.3.92 GS21 Sprayed Panther (IPU + diflufenican) herbicide

14.5.92 GS 30 107 kg/ha nitrogen (as urea)

APPENDIX A4.

Details of husbandry appertaining to all plots in Experiment WKCL 2.

Location. Large Marsh field, Harper Adams Agricultural College, Newport, Shropshire.

The following treatments were applied to all plots.

22.10.92 Drilled (depth 3.5cm) Seed rate 400 seeds/m^2

Cultivar Riband & Apollo Dressing S.P.D.

4.2.93 Isoproturon herbicide 3.3 l/ha

9.3.93 116 kg/ha ammonium nitrate (34.5%N)

26.4.93 231 kg/ha ammonium nitrate (34.5%N)

Appendix B1. Mean colony growth of S. nodorum in culture media containing different levels of added urea (Experiment SV2.)

	STRAIN 171			STRAIN 465		
UREA CONC. % w/v	DAY 4	DAY 7	DAY10	DAY 4	DAY 7	DAY 10
0	7.5	10.2	23.5	35.1	30.8	35.1
1	9.7	9.0	12.7	8.8	8.6	8.8
2	8.0	8.5	10.3	9.4	6.8	9.4
3	4.4	5.0	4.9	3.9	1.0	4.2
5	4.0	4.1	4.2	4.2	4.2	4.2
6	4.0	4.0	4.0	4.0	4.0	4.0
7	4.0	4.0	4.0	4.0	4.0	4.0
8	4.0	4.0	4.0	4.0	4.0	4.0

S.E.M			
Strain	0.30	3.4	0.70
Urea	0.42	4.8	0.99
Strain*urea	0.59	6.8	1.40

ANOVA			
Strain	NS	NS	*
Urea	***	***	***
Linear	***	***	***
Quadratic	***	NS	***
Cubic	NS	NS	***
Strain * urea	NS	NS	NS
Dev. Linear	NS	NS	NS
Dev. Quadratic	NS	NS	NS
Dev.Cubic	NS	NS	NS
CV%	33.0	124.9	24.0
d.f.	31(1)		

Standard error and anova based upon levels 0 - 3% only.

Appendix B2 Experiment GH4a. The effect of foliar sprays of KCl or water or a soil drench of KCl applied at four times relative to inoculation on the percentage area of the upper side of the third leaf from the stem base of winter wheat cv. Apollo affected by *E. graminis*.

Upper side

form	soil	foliar	water	
	4.77	1.62	4.73	
time/days	+7	+3	-3	-7
	4.13	3.11	3.34	4.23
time/days	+7	+3	-3	-7
form				
soil	7.16	3.60	4.30	4.00
foliar	0.92	1.12	2.32	2.10
water	4.30	4.62	3.41	6.60

S.E.M.	form	time	form*time
	0.716	0.827	1.432

C.V. 112
d.f. 96(3)

ANOVA	
Application	***
Timing	NS
Linear	NS
Quadratic	NS
Cubic	NS
Application * Timing	NS
Linear*dev	NS
Quadratic*dev	NS
Cubic*dev	NS

Appendix B3 Experiment GH4a. The effect of foliar sprays of KCl or water or a soil drench of KCl applied at four times relative to inoculation on the percentage area of the lower side of the third leaf from the stem base of winter wheat cv. Apollo affected by *E. graminis*.

Lower side

form	soil	foliar	water	
	3.29	1.05	3.53	
time/days	+7	+3	-3	-7
	1.45	2.69	2.44	3.90
time	+7	+3	-3	-7
form				
soil	1.52	5.41	2.11	4.10
foliar	0.71	0.36	1.01	2.11
water	2.12	2.30	4.20	5.50

S.E.M.	form	time	form*time
	0.494	0.570	0.988

C.V. 119 d.f. 96(3)

ANOVA	
Timing	NS
Linear	NS
Quadratic	NS
Cubic	NS
Application * Timing	NS
Linear*dev	NS
Quadratic*dev	NS
Cubic*dev	NS

Appendix B4. Experiment GH4b. The effect of foliar sprays of KCl or water applied at four times relative to inoculation on the percentage area of the upper and lower sides of the third leaf from the stem base of winter wheat cv. Apollo.

Upper side

form	foliar	water
	1.12	5.56

time/days	+7	+3	-3	-7
foliar	0.9	0.3	1.9	1.3
water	5.8	5.9	3.4	7.1
mean	3.4	3.1	2.7	4.2

S.E.M.	form	time	form*time
	0.99	0.70	1.40

ANOVA

Application	***
Timing	NS
linear	NS
Quadratic	NS
Cubic	NS
Application*Timing	NS
Linear*Dev	NS
Quadratic*Dev	NS
Cubic.Dev	NS
d.f.	64
C.V.	132.5

Appendix B5. Experiment GH5. The effect of potassium chloride and polyethylene glycol solutions with different osmotic potential applied to wheat plants cv. Apollo on the leaf area affected by *E. graminis*.

	LEAF 3 upperside	LEAF 3 lowerside	LEAF 4 upperside	LEAF 4 lowerside
Control	12.400	4.764	14.240	5.280
water	8.804	3.160	10.640	3.920
KCl 12.83 bar	4.600	1.684	6.720	2.248
KCl 25.65 bar	3.420	1.232	6.280	1.952
KCl 38.48 bar	2.764	1.132	4.128	1.412
KCl 51.31 bar	2.200	0.756	4.008	1.352
KCl 64.14 bar	0.936	0.304	1.932	0.632
PEG 12.83 bar	4.760	1.940	8.640	2.480
PEG 25.65 bar	4.167	1.592	8.375	2.542
PEG 38.48 bar	3.171	1.288	6.708	2.713
PEG 51.31 bar	2.920	1.672	4.129	1.013
PEG 64.14 bar	2.550	0.871	4.083	1.496
ANOVA				
BLOCK	***	***	***	***
Treatment	***	***	***	***
Osmotic	***	*	***	***
Linear	***	***	***	***
Quadratic	NS	NS	NS	NS
Cubic	NS	NS	NS	NS
Deviations	NS	NS	NS	NS
Source	NS	NS	NS	NS
Osmotic*Source	NS	NS	NS	NS
Linear*Dev	NS	NS	NS	NS
Quadratic*Dev	NS	NS	NS	NS
Cubic*Dev	NS	NS	NS	NS
Deviations	NS	NS	NS	NS
S.E.M.				
Treatments	0.636	0.334	0.701	0.382
Osmotic	0.450	0.236	0.496	0.270
Source	0.284	0.149	0.314	0.171
Osmotic*Source	0.636	0.334	0.701	0.382
M.S.D.	0.395	O.207	0.436	0.238
d.f.	161	161	161	161
C.V.	72.50	72.50	56.50	88.00

Appendix B6. Experiment GH5. Parallel regression of the mean values for spore germination and percentage leaf area affected by *E. graminis* in response to the application of solutions of different osmotic potential expressed as a percentage of the 0 bar value.

Leaf area affected

	Estimate	S.E.
Intercept	87.22	3.80
Slope	-0.917	0.09

Percentage variance accounted for 96.3

Percentage spore germination

	Estimate	S.E.
Intercept	85.51	7.21
Slope	-0.617	0.17

Percentage variance accounted for 75.4

Anova	d.f.	Significance
Overall	1	***
Position	1	**
Regression	1	NS
Residual	6	
Total	9	

Appendix B7. Experiment UP1. The depletion of potassium ions from the leaf surface of winter wheat (cv. Apollo) following the application of potassium chloride in controlled quantities.

	potassium concentration (ppm)
Control (pre treatment)	2.40
0 hours	33.19
3 hours	17.73
6 hours	14.50
12 hours	9.36
24 hours	10.02
48 hours	6.32
ANOVA	
Block	NS
Ttreatment	***
Time	***
Linear	***
Quadratic	***
Cubic	NS
Deviations	NS
S.E.M.	2.298
d.f.	78
CV.	58.4

Appendix B8. Experiment UP1. The depletion of chloride ions from the leaf surface of winter wheat (cv. Apollo) following the application of potassium chloride in controlled quantities.

	chloride concentration (ppm)
Control (pre treatment)	4.8
0 hours	207.1
3 hours	51.5
6 hours	44.0
12 hours	12.9
24 hours	19.9
48 hours	12.4
Anova	
Block	NS
Treatment	***
Time	***
Linear	***
Quadratic	***
Cubic	*
Deviations	NS
S.E.M.	10.62
d.f.	78
CV.	74.8

Appendix B9. Experiment UP1. The depletion of polyethylene glycol from the leaf surface of winter wheat (cv. Apollo) following the application of polyethylene glycol in controlled quantities.

	polyethylene glycol conc. (ppm)
Control (pre treatment)	0.224
0 hours	2.253
3 hours	1.338
6 hours	1.361
12 hours	0.889
24 hours	0.654
48 hours	0.512
ANOVA	
Block	NS
Treatment	***
Time	***
Linear	***
Quadratic	***
Cubic	*
Deviations	NS
S.E.M.	0.232
d.f	78
CV.	75

Appendix B 10. Experiment LWP 3. Analysis of variance of the differences in leaf water potential of wheat cv. Apollo, treated with foliar sprays of either water or potassium chloride solution, over time (bar).

solution	potassium chloride	water	mean
time/hours			
0	14.33	13.67	14.00
24	15.08	11.50	13.29
72	14.17	12.33	13.25
168	12.83	8.67	10.75
312	10.83	10.50	10.67
mean	13.45	11.33	

S.E.M	
time	0.393
solution	0.249
time*solution	0.556

ANOVA	
Time	***
Linear	***
Quadratic	NS
Cubic	NS
Solution	**
Time*Solution	NS
Linear*Dev	**
Quadratic*Dev	NS
Cubic*Dev	NS
d.f.	49
c.v. %	11

Appendix B11. Experiment REG1. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the percentage area of the upper surface of the fourth leaf from the stem base affected by *E. graminis*.

osmoticum	PEG	KCl	mean
osmotic potential			
12.83	5.70	3.80	4.75
25.65	3.68	2.50	3.09
38.48	2.41	3.30	2.86
51.31	1.03	1.03	1.03
64.14	0.55	0.50	0.53
mean	2.67	2.23	

control 7.40 water 6.70

S.E.M	0.495
ANOVA	
Block	NS
Osmotic potential	***
Linear	***
Quadratic	NS
Cubic	NS
Deviations	*
Osmotica	NS
Osmotica*Osmotic potential	*
Linear*Dev	*
Quadratic*Dev	NS
Cubic*Dev	NS
Deviations	NS
d.f.	90
C.V.%	52.0

Appendix B12. Experiment REG1. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the percentage area of the lower surface of the fourth leaf from the stem base affected by *E. graminis*.

osmoticum	PEG	KCl	mean
osmotic potential			
12.83	4.20	4.10	4.15
25.65	1.98	2.40	2.19
38.48	0.99	1.42	1.20
51.31	0.40	0.70	0.55
64.14	0.55	0.50	0.53
mean	1.62	1.82	
control 6.00 water 5.90			
S.E.M	0.586		
ANOVA			
Osmotic potential	***		
Linear	***		
Quadratic	NS		
Cubic	NS		
Deviations	NS		
Osmotica	NS		
Osmotica*Osmotic potential	***		
Linear*Dev	NS		
Quadratic*Dev	NS		
Cubic*Dev	NS		
Deviations	NS		
d.f.	90		
C.V. %	67.9		

Appendix B13. Experiment REG1. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the leaf water potential the fourth leaf from the stem base (bar).

Osmotica	PEG	KCl	mean
Osmotic Potential			
12.83	-12.15	-12.05	-12.10
25.65	-13.15	-12.92	-13.05
38.48	-13.80	-13.10	-13.45
51.31	-14.15	-11.70	-14.43
64.14	-14.55	-16.02	-15.29
mean	-13.56	-13.77	
control -10.3 water -8.90			
S.E.M	0.340		
ANOVA			
Osmotic potential	***		
Linear	***		
Quadratic	NS		
Cubic	NS		
Deviations	*		
Osmotica	NS		
Osmotica*Osmotic potential	*		
Linear*Dev	*		
Quadratic*Dev	*		
Cubic*Dev	NS		
Deviations	NS		
d.f.	90		
C.V.%	8.3		

Appendix B14. Experiment REG1. Multiple linear regression of the mean values for leaf water potential and percentage area of the fourth leaf from the stem base affected by *E. graminis* in response to the application of solutions of different osmotic potential.

Regression Analysis.

Summary of analysis.

	df	ss	ms	vr
regression	20	229.2	11.462	7.56
residual	73	110.7	1.5616	
total	93	339.9	3.655	

change

variance accounted for 58.5%

Estimates of regression coefficients.

	estimate	s.e.	t
constant	3.25	2.96	1.10
Block 2	0.368	0.612	0.6
Block 3	0.391	0.560	0.70
Block 4	0.444	0.592	0.75
Block 5	0.017	0.581	0.03
Block 6	0.910	0.590	1.54
Block 7	1.423	0.589	2.41
Block 8	-0.073	0.601	-0.02
Block 9	0.053	0.572	0.09
Block 10	0.143	0.598	0.24
Osmotica	-2.10	3.750	-0.56
LWP	-0.141	0.243	-0.58
LWP*.Osmotica	-0.048	0.305	-0.16
Osmotic Potential 25.65	-1.775	0.638	-2.78
Osmotic Pot 38.48	-3.145	0.682	-4.61
Osmotic Potential 51.31	-4.480	0.763	-5.86
Osmotic Potential 64.14	-4.982	0.850	-5.86
Osmotica*Osmotic Potential*25.65	0.305	0.857	0.36
Osmotica*Osmotic Potential*38.48	2.446	0.893	2.74
Osmotica*Osmotic Potential*53.31	1.21	1.06	1.14
Osmotica*Osmotic Potential*64.14	1.06	1.20	0.89

Appendix B 14 cont.

ACCUMULATED ANALYSIS OF VARIANCE.

Change	d.f.	significance
+Block	9	NS
+Source	1	NS
+LWP	1	***
+LWP*Osmotica	1	*
+Osmotic Potential	4	***
Osmotica*Osmotic Potential	4	*
Residual	73	
TOTAL	93	

Appendix B15. Experiment REG2. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the germination of *Erysiphe graminis* spores on the upper side of the fourth leaf from the stem base.

Osmotica		PEG	KCl	mean
Osmotic Potential				
12.83		0.5470	0.3783	0.4625
25.65		0.3260	0.2870	0.3065
38.48		0.2750	0.2780	0.2765
51.31		0.1754	0.1650	0.1702
64.14		0.1814	0.1060	0.1437
mean		0.3010	0.2428	
control 0.8760	water 0.6830			
S.E.M		0.02745		
ANOVA				
Osmotic Potential	***			
Linear	***			
Quadratic	*			
Cubic	NS			
Deviations	*			
Osmotica	**			
Osmotica*Osmotic Potential	*			
Linear*Dev	NS			
Quadratic*Dev	**			
Cubic*Dev	NS			
Deviations	NS			
d.f.	79			
C.V.%	24.3			

Appendix B16. Experiment REG2. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the leaf water potential of the fourth leaf from the stem base (bar).

Osmotica		PEG	KCl	mean
Osmotic Potential				
12.83		-10.48	-10.23	-10.35
25.65		-11.15	-11.88	-11.51
38.48		-12.15	-11.20	-11.68
51.31		-14.65	-12.10	-13.38
64.14		-15.20	-12.15	-13.68
mean		-12.73	-11.51	
control -8.38	water -8.20			
S.E.M	0.509			
ANOVA				
Osmotic Potential	***			
Linear	***			
Quadratic	NS			
Cubic	NS			
Deviations	**			
Osmotica	***			
Osmotica*Osmotic Potential	**			
Linear*Dev	***			
Quadratic*Dev	NS			
Cubic*Dev	NS			
Deviations	*			
d.f.	79			
C.V.%	14.0			

Appendix B17. Experiment REG2. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the percentage area of the upper surface of the fourth leaf from the stem base affected by *E. graminis*.

Osmotica		PEG	KCl	mean
Osmotic Potential				
12.83		5.80	5.40	5.60
25.65		3.60	3.90	3.75
38.48		2.50	1.57	2.04
51.31		1.70	1.40	1.55
64.14		2.00	0.45	1.22
mean		3.12	2.54	
control 10.4	water 8.60			
S.E.M	1.122			

ANOVA	
Osmotic Potential	**
Linear	***
Quadratic	NS
Cubic	NS
Deviations	NS
Osmotica	NS
Osmotica*Osmotic potential	NS
Linear*dev	NS
Quadratic*dev	NS
Cubic*dev	NS
Deviations	NS
d.f.	79
C.V.%	90.5

Appendix B18. Experiment REG2. The effect of potassium chloride and polyethylene glycol solutions at different osmotic potentials applied to wheat plants cv. Apollo on the percentage area of the lower surface of the fourth leaf from the stem base affected by *E. graminis*.

Osmotica		PEG	KCl	mean
Osmotic Potential				
12.83		4.66	5.70	5.18
25.65		5.00	4.70	4.70
38.48		2.77	3.70	3.70
51.31		2.40	3.00	3.00
64.14		1.70	2.00	2.00
mean		3.31	3.82	
control 9.90	water 9.20			
S.E.M	1.188			
ANOVA				
Osmotic Potential	*			
Linear	**			
Quadratic	NS			
Cubic	NS			
Deviations	NS			
Osmoticum	NS			
Osmoticum*Osmotic Potential	NS			
Linear*Dev	NS			
Quadratic*Dev	NS			
Cubic*Dev	NS			
Deviations	NS			
d.f.	79			
CV%	82.5			

Appendix B19. Experiment REG2. Multiple linear regression of the mean values for spore germination, leaf water potential and percentage area of the fourth leaf from the stem base affected by *E. graminis* in response to the application of solutions of different osmotic potential.

Regression Analysis

Estimates of regression coefficients

	estimate	s.e.	t(75)
Constant	15.53	5.07	3.06
Block 2	-4.86	1.56	-3.11
Block 3	-6.85	1.66	-4.13
Block 4	-6.63	1.59	-4.18
Block 5	-5.05	1.61	-3.14
Block 6	-6.48	1.57	-4.13
Block 7	-5.09	1.57	-3.23
Block 8	-7.10	1.61	-4.41
Block 9	-6.49	1.56	-4.15
Block 10	-5.21	1.53	-3.40
Osmoica PEG	-6.18	7.18	-0.86
Spore germination	-0.1140	0.0638	-1.79
Spore germination* Osmotica PEG	0.1876	0.0899	2.09
LWP	0.041	0.364	0.11
LWP*Osmotica PEG	-0.136	0.539	-0.25
OP 25.65	-1.97	2.01	-0.98
OP 38.48	-4.65	2.28	-2.04
OP 51.31	-6.34	3.19	-1.98
OP 64.14	-7.25	3.36	-2.16
Osmotica PEG .OP 25.65	1.76	2.62	0.67
Osmotica PEG .OP 38.48	3.43	2.81	1.22
Osmotica PEG .OP 51.31	5.30	3.78	1.40
Osmotica PEG .OP 64.14	5.65	4.06	1.39

Appendix B19 cont.

Accumulated analysis of variance

Change	d.f	
+ Block	9	***
+ Osmotica	1	NS
+ Spore germination	1	*
+ Spore germination*Osmotica	1	NS
+ LWP	1	NS(p=0.01)
+ LWP.Osmotica	1	NS
+ OP	4	NS
+ Osmotica.OP	4	NS
Residual	75	
Total	97	